Influence of Topically Applied Adrenergic Agents on Cochlear Blood Flow

K. Agnetha Ohlsen, David L. Baldwin, Alfred L. Nuttall, and Josef M. Miller

This study was designed to assess the role of adrenergic receptors in the control of cochlear blood flow. Laser Doppler flowmetry was used to determine the effects of adrenergic drugs topically applied to the round window membrane of the cochlea. The relative influence of the various receptor types ($\alpha_1$, $\alpha_2$, $\beta_1$, and $\beta_2$) was examined by a selection of agonists and antagonists. The agonists norepinephrine and epinephrine, which have mixed $\alpha$- and $\beta$-receptor effects, and phenylephrine, a strong $\alpha_1$-agonist, all induced a dose-dependent reduction in cochlear blood flow. The agonists isoproterenol ($\beta$-active), salbutamol ($\beta_2$-active), and BHT 933 ($\alpha_2$-active) had no effect on cochlear blood flow. Of the antagonists, when tested alone, only the selective $\alpha_1$-antagonist prazosin had a direct effect on cochlear blood flow, demonstrating an increase in cochlear blood flow. The selective $\alpha_1$-antagonist idazoxan, the $\beta$-antagonist propranolol, and the unselective $\alpha$-antagonist phentolamine had no effect on cochlear blood flow. Interaction studies of agonists and antagonists were performed to specifically define the receptor subclasses responsible for the cochlear blood flow increases with norepinephrine and epinephrine. The results are consistent with the presence of an $\alpha_\text{adrenergic sympathetic control of cochlear blood flow.} (Circulation Research 1991;69:509–518)

The autonomic innervation of the cochlea has been the subject of many anatomic, physiologic, and pharmacological investigations and reviews. A variety of histological techniques, including histochemistry, 1-4 light and electron microscopy, 5 Golgi's stain, 6 and autoradiography, 7 have provided strong evidence for the existence of adrenergic fibers within the cochlea. Moreover, these studies describe their anatomic distribution in detail.

Two types of adrenergic fibers have been identified 1-4, 8; those arising bilaterally from the stellate ganglia, which terminate directly on blood vessels, and those derived unilaterally from the respective ipsilateral superior cervical ganglion, which do not end on blood vessels. The role of the nonperivascular fibers is a subject of some debate. 9,10 They are anatomically associated with auditory afferent neurons, and Hultcrantz et al 11 found altered sensitivity to sound after an ipsilateral cervical sympathectomy. The role and the specific influence of fibers that end on cochlear blood vessels also is not clear. It is assumed that at least part of their function is to control cochlear blood flow (CBF).

Studies of the effects of sympathetic stimulation and transection 12-14 and systemic administration of adrenergic agents 15 have yielded controversial results. Although some of the investigations indicate that this system may mediate an autonomic control of CBF, this control is weak and easily overcome by systemic blood pressure (BP) changes. 15-17 Other studies 18-20 have shown that CBF may vary independent of systemic BP, indicating the presence of locally controlled factors. These sympathetic and other local influences may act to maintain a stable flow and an optimal homeostatic environment in this highly active and metabolically sensitive organ. 21,22 Such local influences are consistent with the hypothesized, but yet to be confirmed, proposed autoregulation control of CBF. 14,23,24 Thus, on the basis of current data, it may be argued that CBF is a complex product of many factors, including systemic BP, local metabolic agents, locally active circulating hormones, and the sympathetic system.

Sympathetic fibers to the cochlea release neurotransmitter to available receptors. Presumably, these also could be activated by administered adrenergic drugs. The resultant effect, vasodilation or contraction, depends on the varying proportion and density of receptor subtypes in the tissue and the concentration of the agent.

One way to evaluate the potential role of sympathetic control of CBF is to inventory the types and assess the functional level of adrenergic receptors in...
the cochlea. Thus, it was the goal of this study to carry out this survey under conditions of stable systemic cardiovascular physiology. To avoid the influence of systemic variables, this investigation used topical application of putatively active agents at drug receptor sites. This was accomplished by applying the agents to the fenestra tympani (the round window of the cochlea). CBF was assessed using laser Doppler flowmetry.

**Materials and Methods**

**Animals and Preparation**

Approximately 80 pigmented guinea pigs of either sex, weighing 250–480 g, were used in this investigation. All subjects had a normal Preyer reflex (the startle reflex movement of the auditory pinnae with sound) and were free of middle ear infection, as assessed directly under the operating microscope. Each guinea pig was anesthetized with 15 mg/kg i.p. pentobarbital sodium followed 15 minutes later by 0.4 ml/kg i.m. Innovar-Vet (Pitman-Moore, Mundelein, Ill.). A stable anesthesia level was maintained by an additional half-dose injection of Innovar-Vet every hour and a half-dose injection of pentobarbital sodium every 2 hours. The anesthesia combination has been shown to be reliable for maintaining a normal systemic BP.25 Lidocaine-HCl (1%) was injected, if necessary, at the site of skin incisions. Core body temperature, measured with a rectal probe, was maintained at 38°C with a thermoregulated heating blanket. The head was placed in a heated head-holder, which positioned the guinea pig for CBF measurements while preventing conductive cooling of the head. Tracheotomies were performed to ensure an adequate airway, and the guinea pigs were self-respiring in all experiments. The right carotid artery, contralateral to the measured ear, was cannulated (Micro-Renathane catheter tubing) to monitor systemic BP, and the right external jugular vein was cannulated (PE-50 catheter) when studies required intravenous administration of drugs.

A ventrolateral surgical approach was used to expose the left auditory bulla. The tympanic membrane and ossicles remained intact in all preparations, and the bulla defect was enlarged sufficiently to expose the entire cochlea. The mucosa and periosteum overlying the otic capsule were carefully removed using a cotton pledget. In some cases, both ears were exposed for simultaneous CBF study.

**CBF Recording**

CBF was monitored by a laser Doppler flowmeter (Laser Flow Blood Perfusion Monitor 403, TSI, Vasamedics, Inc., St. Paul, Minn.). The probe (0.7-mm diameter) was placed over the lateral wall of the first or second turn of the cochlea. A light petroleum jelly was used as light transmission gel (between the probe and the cochlea), and all recordings were done under stable illumination from the surgical microscope. A second laser Doppler flowmeter (model LD5000, Med Pacific) was used alternately to monitor skin blood flow or, for comparison, unmanipulated contralateral ear blood flow. In the cases where recordings from both ears were done, the two laser Doppler flowmeters were used alternately on the left or right side. Moreover, before use in this experiment, the output of the two flowmeters was compared on skin with increasing doses of intravenous epinephrine. The measured percentage of change of CBF was identical for the two instruments.

Laser Doppler flowmetry provides a voltage output proportional to the product of the number of red blood cells in the measurement volume and their velocities, that is, flux. However the calibration of the instrument in physical units of blood flow has not been possible for the inner ear; thus, in this study, all changes in CBF are expressed as relative change in blood flow from an initial baseline. More details of laser Doppler flowmetry have been published elsewhere.26

**Topical Drug Application**

To assess the influence of adrenergic drugs on CBF, while avoiding systemic BP alterations, certain mass quantities of agents were applied topically to the round window membrane of the cochlea (RW) in vehicle volume amounts of 0.4–1.5 μl by a microsyringe. This approach takes advantage of the concentration-dependent passage of small molecular weight substances through the intercellular spaces in a normal RW.27–31 All drugs tested in this investigation were <500 Da, which was within the range shown in previous investigations to diffuse readily through the RW. It must be emphasized that this approach does not expose the inner ear vasculature; the cochlea is intact and in its normal state. Although the exact site of action of vasoconstrictors on smooth muscle in the cochlea is not known when these drugs pass through the RW, it is possible that the vascular effects occur in the spiral modiolus artery. The spiral modiolus artery (the main cochlear vessel) is within the central core of the cochlea, a diffusion distance of ~1 mm from the site of drug application. Vasoconstriction of the spiral modiolus artery will lead to altered blood flow throughout the cochlea.

**Experimental Protocol**

Two experimental protocols were followed in this study. The first was used to determine the presence of cochlear adrenergic receptors, define the effects, and obtain dose–response curves for each topically applied agonist. The second protocol was used to assess drug interactions.

**Protocol 1.** A 15-minute baseline measure (BL1) was made first, and subsequent steps were followed only if BL1 demonstrated a stability of a few percent. This was followed by a 5-minute observation of the effects of the vehicle associated with the drug, either saline, sterile water, or 5% ethanol in sterile water. Then the influence of the experimental drug (agonist) on CBF was assessed, with a measurement period that varied, depending on the observed time course of effect of the agent. In most cases, the drug
remained on the RW until the CBF returned to baseline, but in some instances, the effect of drug removal was assessed. Finally, a second baseline measurement (BL2) was made, this time for 2 minutes. When an agent was tested at a single concentration, the sequence (vehicle, drug, BL2) was repeated at least four times, and the maximum response values were averaged. When a dose–response function was determined for a particular agent (in a single guinea pig), the same sequence was used but with only one or two applications of each concentration level. The order of concentration application was randomized.

**Protocol 2.** The second protocol tested agonist/antagonist interactions. It consisted of two repetitions of the sequence used in protocol 1 to assess the effect and repeatability of a selected agonist. This was followed by assessment of the influence of an antagonist on the agonist-elicited CBF response by repeating the sequence (vehicle, drug, BL2) after application of the antagonist. Antagonists used for these interaction studies were selected for properties that would permit specific receptor differentiation, that is, high potency, high degree of selective affinity for individual receptor subtypes, and lack of intrinsic sympathomimetic activity (i.e., efficacy). These antagonists were phentolamine ($\alpha_1$ and $\alpha_2$), prazosin ($\alpha_1$), idazoxan ($\alpha_2$), and propranolol ($\beta_1$ and $\beta_2$).32–36

All antagonists were first tested alone in the same way as the agonists by following protocol 1. This not only determined their own effects on CBF but also explored the possible release of sympathetic tone. Interactions were studied between the two agonists norepinephrine and epinephrine and each antagonist by following protocol 2.

Although drugs were usually administered topically via the RW, on occasion, as indicated in “Results,” the antagonist was administered intravenously as well. The interval between antagonist administration and agonist application was based on the time course of antagonist effects. Pilot observations indicated that the time courses reported by Goodman and Gilman37 were appropriate for the cochlea, for both topical and systemic application. In each case, the agonist was administered when any antagonist-induced response had stabilized. The dose of the antagonist was selected at levels relative to the effective dose of the agonist and known from the literature to produce a significant blocking effect.16,37

In cases in which solubility limited the concentration of the antagonist and the size of the RW niche limited the volume, repeated application of the drug was given to reach the required effective dose.

In this study, dosage of all drugs applied to the RW is given as total weight of active drug (base). Although dose specification as a percentage in solution would be possible if the total volume applied were held constant, drug solubilities (for dose–response functions) made it more practical to vary both the volume applied and the drug concentration in that volume. This unusual situation is mandated by the small volumes that can be applied to the RW and the fact that the internal fluid volume of the cochlea dominates over the volume that can be applied.

Since little evidence for the presence of $\alpha_2$-receptors was observed in this study, the dose–response functions for epinephrine and norepinephrine were defined before and after administration of the selective $\alpha_1$-antagonist prazosin. Specifically, for a given experiment, following protocol 1, a dose–response function was obtained for one of the topically applied agonists alone at drug weights of 2–110 $\mu$g for epinephrine and 2–90 $\mu$g for norepinephrine. Prazosin was then applied to the RW. Because of low drug solubility, this was accomplished by three applications of 33 $\mu$g in 1 $\mu$l vehicle. Each application remained on the RW for ~10 minutes. This was followed by the second measurement of the agonist dose–response function. Concentrations of the agonist were administered in a random fashion. All concentrations were tested at least twice in each guinea pig, and dose–response curves of the maximum response to the drug were plotted.

In addition to the RW application of prazosin, some guinea pigs received 22 $\mu$g/kg i.v. prazosin. This caused a higher degree of competitive receptor blocking than was possible by use of the RW route alone. In humans, this intravenous dose of prazosin produces a decrease in BP of <5%. With this combined topical and intravenous administration, instead of intravenous administration alone, a high level of blocking effect was reached while avoiding changes in BP.

To assess the effect of complete receptor blockade, the irreversible antagonist phenoxybenzamine was used. Epinephrine, observed to be the most powerful $\alpha$-agonist, was tested before and after phenoxybenzamine administration. This antagonist was administered in a slow intravenous infusion, 1 mg/kg for 1 hour, to allow maximum fully irreversible covalent binding while avoiding other systemic reactions. Dose–response functions were determined for epinephrine applied topically to the RW and systemically (intravenously) before and after phenoxybenzamine administration by following protocol 2.

Recovery of baseline function after an adrenergic agonist-elicited response is dependent on elimination of the agonist from the receptor. Different mechanisms play a role in this elimination. The primary factor is reuptake into the nerve terminal and metabolism. Inhibition of reuptake should therefore lead to a prolonged agonist-elicited response. In other systems, tricyclic antidepressants are well-known reuptake inhibitors of catecholamines.37 Hence, the effect of the antidepressant imipramine on a norepinephrine-elicited CBF change was examined. For this study protocol 2 was followed: repeat observations were made of a norepinephrine-elicited decrease in CBF, followed by administration of imipramine (2 mg/kg i.v.) and repeat application of norepinephrine 45 minutes later. The dose of imipramine was based on guidelines for medical treatment and represents the highest daily intake recom-
mended without inducing significant side effects. The drug was administered intravenously to permit a first-pass effect by the liver producing the active metabolite desmethylimipramine.

Throughout all of the above experiments CBF and BP were monitored continuously, and measurement of skin blood flow or contralateral CBF was made frequently.

Results

Vehicles

Administration of the vehicle, either saline, sterile water, or 5% ethanol in sterile water, when applied to the RW topically or when given intravenously resulted in no significant change in CBF.

Agonists

In the presence of stable BP, topically applied epinephrine, norepinephrine, and phenylephrine caused a concentration-dependent decrease in CBF.

Epinephrine. Topical RW application of this mixed α1, α2, and β-agonist was studied in 30 guinea pigs for drug weights (mass) of 1–110 μg. Concentrations <4 μg were mostly ineffective. Figure 1 illustrates a typical ipsilateral CBF response to epinephrine. Note that contralateral CBF and systemic BP remained stable. The latency of CBF reduction was <1.5 minutes after administration. Subsequently, there was a fairly rapid decrease in CBF to the maximum flow reduction elicited by each concentration and then a two-component recovery consisting of an initial rapid recovery followed by a slower component. The duration of effect was 13±3 minutes for all but two guinea pigs, for which the effect lasted 20 and 22 minutes. These effects were repeatable within subjects with rather small differences. In five of the 30 guinea pigs studied, a 15–20% recovery overshoot was observed, which lasted 5–11 minutes before final return to predrug baseline. Six guinea pigs showed no response to topical epinephrine administration yet showed a normal increase in BP and CBF with systemic administration. One guinea pig showed no recovery after CBF reduction.

Figure 2 illustrates the pattern of change when topically applied epinephrine was dried from the membrane by a piece of absorbent tissue. Mean curves for a low and high concentration of epinephrine are shown; the response when the drug was allowed to remain on the RW is compared with the response when the drug was removed. After drug removal, recovery was complete within 2–5 minutes. In these examples, the concentration-dependent nature of the response is also evident.

Figure 3A shows the dose–response CBF function obtained for topically applied epinephrine. A saturating response was produced at higher doses, causing the baseline CBF to be reduced by ~40–50%. The half-maximal response was at ~25 μg. The closed symbols in the figure are data obtained from a single dose of epinephrine in individual guinea pigs. The open symbols are data from multiple guinea pigs (dose–response functions within animals). The two sets of data can be justifiably pooled, and least squares are fitted with a third-order function.
Norepinephrine. RW application of norepinephrine, with α₁, α₂, and low β-adrenergic properties, was studied in 28 guinea pigs. It demonstrated qualitative effects similar to epinephrine but of smaller magnitude, as shown in Figure 3B. The fitted sigmoidal functions of Figures 3A and 3B are approximately parallel, and an equal weight of norepinephrine gives about one half the effect caused by epinephrine. The time course of effect of single doses was 12±2 minutes; however, in two guinea pigs it lasted 20 minutes, and in one it lasted 22 minutes. Recovery was in two phases; as with epinephrine, an initial rapid approximately one-third recovery was followed by a slower component. No recovery overshoot was observed. One guinea pig showed no recovery; in seven, no response to topical administration was observed, and only two of these were responsive to systemic administration.

Phenylephrine and other agonists. Topical application of the α₁-agonist phenylephrine caused a dose-dependent reduction in CBF without a change in BP in nine of the 12 guinea pigs studied. The drug was tested at drug weights ≤120 μg and elicited a maximal reduction in CBF of ~25%. The latency of onset showed a greater variation than for epinephrine and norepinephrine, but all responsive guinea pigs showed a reduction within 2.5 minutes, followed by a rapid continuous decrease to a maximum reduction. Recovery followed the pattern observed for norepinephrine and epinephrine. Full recovery was achieved by 36–48 minutes after administration. Removal of the drug from the RW did not change the course of recovery but did limit CBF reduction. Six guinea pigs did not show full recovery. Three guinea pigs showed no response to the topically applied agent but did respond to systemic administration.

No change in CBF was elicited with topical application of a 1.5-μl volume at 5% concentrations of the α₂-agonist, BHT 933 (n=4), the unselective β-agonist, isoproterenol (n=5), or the β₂-selective agonist, salbutamol (n=4).

Antagonists

Topical application of 100 μg (three applications of 33 μg/μl) of the α₁-selective antagonist prazosin induced a slow increase of 14–45% in CBF in four of the six guinea pigs. None showed recovery after 2 hours.

Topical application (1.5-μl volumes) of α₁- and α₂-agonist (10% phentolamine, n=3), selective α₂-agonist (10% idazoxan, n=4), or β₁- and β₂-agonist (10% propranolol, n=5) demonstrated no significant change in recorded CBF.

Drug Interactions

Table 1 shows the results of interaction tests for epinephrine and norepinephrine when antagonized by prazosin (α₁-antagonist), phentolamine (α₁- and α₂-antagonist), idazoxan (α₂-agonist) and propranolol (β₁- and β₂-agonist). To accomplish this experiment, the CBF decrease elicited by a half-maximal

<table>
<thead>
<tr>
<th></th>
<th>Prazosin</th>
<th>Phentolamine</th>
<th>Idazoxan</th>
<th>Propranolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>57±12.5</td>
<td>6</td>
<td>40±19.4</td>
<td>4</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>85±30</td>
<td>4</td>
<td>67±13.7</td>
<td>2</td>
</tr>
</tbody>
</table>

n, Number of guinea pigs.
effective topical dose of epinephrine and norepinephrine was compared with that obtained in the presence of the antagonist. The table expresses the percentage difference, where 100% would indicate complete blockage. Administration of 100 μg prazosin on the RW reduced the influence of CBF by ~57% and reduced the response to norepinephrine by 85%. Administration of 10% phenolamine (1.5-μl volume) to the RW decreased the effectiveness of the subsequent application of epinephrine by 40% and norepinephrine by an average 67%.

Topical application of 1.5-μl volumes of 10% idazoxan and 5% propranolol had no significant effect on the CBF response elicited by epinephrine or norepinephrine. Two lower concentrations of each agonist were also examined with no effect on the antagonist. Three observations of each antagonist against each agonist, at each of three agonist concentration levels, were tested in a total of 12 guinea pigs.

The partial block of the altered CBF after topically applied epinephrine in weights of 1–50 μg by 100 μg topically applied prazosin (n=4) is shown in Figure 4A. The figure illustrates dose–response curves from individual guinea pigs for epinephrine before (open symbols) and after (closed symbols) topical antagonist administration. Figure 4B gives dose–response functions for epinephrine before and after administration of 22 μg/kg topical combined with intravenous prazosin (n=2). Topically applied antagonists shift the response curve (Schild plot) to the right in a parallel manner, without significantly affecting the maximal response. This indicates a competitive antagonism. Topical combined with intravenous administration of antagonist evoked a further concentration-dependent parallel shift to the right. This also is likely to be a competitive antagonism, as the depression of the relative maximal response reflects the limit of the tested concentrations of agonist in the presence of antagonist. The BP decreased ~10% after intravenous administration of prazosin.

The dose–response curves for norepinephrine showed effects similar to those obtained for epinephrine. Prazosin caused a rightward shift in the dose–response functions, and the combined topical/intravenous protocol gave a relatively greater blocking effect.

Whereas topical administration of different concentrations of epinephrine caused a concentration-dependent decrease in CBF with stable BP, intravenous injection of epinephrine in four guinea pigs at 0.5, 5, 10, 20, and 50 μg/kg caused a dose-related increase in BP and CBF. Figure 5 shows, for comparison, the dose–response functions of BP (open symbols) and CBF (closed symbols) for intravenously administered epinephrine. This increase in both variables is quite different from that seen with local applications of the drug.

After intravenous administration of the irreversible α1- and α2-antagonist phenoxybenzamine (1 mg/kg), the BP changes and CBF changes to epinephrine administered intravenously in doses from 0.5 μg to 20 μg/kg were blocked. A high test dose of 50 μg/kg i.v. epinephrine elicited a fall in BP of 15.2±2.8 mm Hg in two guinea pigs but caused little change in CBF except for a rapid, small initial dip. The responsiveness to topically applied epinephrine was completely blocked after administration of phenoxybenzamine.

Figure 6 illustrates the effect of pretreatment with imipramine (2 mg/kg i.v.) on the topically elicited norepinephrine decrease in CBF. After administration of this reuptake blocker, the duration for norepinephrine effect was extended 100%, from 14±3.2 to 28.5±4 minutes (n=3).

**Discussion**

Earlier reports have suggested the presence of α-receptors in the cochlear vasculature, based on assessments after systemic administration of drugs.15–17,38,39
Since these data also show a close dependence of CBF on BP, it is difficult to directly study the local mechanisms that may be responsible for controlling CBF. The present experiments, using topical drug applications, have provided an effective approach for the analysis of these local mechanisms that influence CBF. The changes in CBF we observed with administration of catecholamines were elicited without alterations in systemic BP.

The method of using the RW to administer drugs appears appropriate if the molecular weight of the drug is sufficiently small to be readily permeable through the membrane. This was the case in all agents tested in this study. Both direct studies of the physical characteristics of RW permeability and indirect studies on the effects of toxins and pharmacological agents that are used to treat middle ear disease\textsuperscript{27-31} have confirmed that low molecular weight compounds readily cross the normal RW. According to these studies, as summarized by Hamaguchi et al.\textsuperscript{40} agents with a molecular weight <500 should readily be permeable to the RW. The maximum molecular weight of an agent used in this study was 377.

Figure 7 schematically illustrates the topical effect of the various agonists and antagonists tested alone in this investigation as well as their interactions. The data clearly indicate the presence of effective adrenergic receptors in the cochlear vasculature. The left column of Figure 7 indicates that the three powerful adrenergic agonists, norepinephrine, epinephrine, and phenylephrine, given alone (topical application) are highly effective. The response was a dose-dependent reduction in CBF, consistent with an effect dependent on activation of mainly constrictor receptors, that is, postsynaptic $\alpha$-receptors. Topical application of norepinephrine and epinephrine, the agents

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Graph comparing the effect of five different intravenous (IV) doses of epinephrine (0.5, 5, 10, 20, and 50 $\mu$g/kg) on blood pressure (open circles) and cochlear blood flow (filled circles) in four individual guinea pigs.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Graph showing influence of topically applied norepinephrine on cochlear blood flow (CBF) before and after intravenous administration of imipramine, a reuptake blocker.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure7.png}
\caption{Schematic illustration showing effects of agonists and antagonists alone and their interactions on cochlear blood flow.}
\end{figure}
with mixed $\alpha_{1}$-, $\alpha_{2}$-, and $\beta$-receptivity, elicited greater reduction in CBF than the topical application of the selective $\alpha_{1}$ agonist phenylephrine. This could be due to involvement of $\alpha_{2}$-receptors in the response. However, since topical BHT 933, a potent selective $\alpha_{2}$-agonist, exhibited no effect on CBF, it appears that activation of $\alpha_{2}$-receptors by topical epinephrine and norepinephrine cannot explain the lower effectiveness of topical phenylephrine. Similarly, topical studies of isoproterenol and salbutamol indicated no significant influence of $\beta_{1}$-adrenergic inhibitory receptors in these vessels, which would lead to an increased CBF.

Observations on the contribution of $\alpha_{2}$- and $\beta$-receptor influences on the control of CBF were extended through studies of the effect of topically applied $\alpha$- and $\beta$-antagonists. The bottom row in Figure 7 demonstrates the principal outcome after administration with antagonists alone. Only the $\alpha_{1}$-antagonist prazosin had a direct effect on CBF, causing an increase in CBF. The selective $\alpha_{2}$-agonist idazoxan and the selective $\beta$-antagonist propranolol gave no measurable effect on CBF. The mixed $\alpha_{1}$- and $\alpha_{2}$-antagonist phentolamine also demonstrated no effect. Phentolamine has a relatively lower affinity for both $\alpha_{1}$- and $\alpha_{2}$-receptors, which suggests that its concentration in the inner ear failed to reach an effective level using the topical route of administration.

The increase in CBF observed with prazosin supports the view that the vessels are normally under sympathetic tone that prazosin may block. This is consistent with observations of Hultcrantz\textsuperscript{41} demonstrating a 25% increase in CBF with sections of the sympathetic nerves in conscious rabbits. Similarly, the decrease in CBF induced by topical $\alpha$-agonists (Figure 3 and summarized in the left row of Figure 7) may be comparable to the 25% reduction in CBF observed with stimulation of the superior cervical sympathetic ganglion by Hultcrantz\textsuperscript{14} in anesthetized animals. Thus, adrenergic drugs seem to mimic the neurohumoral transmission involved with sympathetic control of vessels in the inner ear, as previously speculated.\textsuperscript{15,42} The study following protocol 1 indicated evidence for presence of adrenergic receptors within the inner ear vasculature but incomplete evidence for specific subclasses.

To specifically define the presence of a receptor subclass, it is appropriate to perform interactive studies in which highly selective antagonists are used to modify the responsiveness of a possible receptor population.

The results of the drug interactions in this study (protocol 2) are also schematically summarized in Figure 7. Topical administration of the $\alpha_{1}$- and $\alpha_{2}$-antagonist prazosin and the mixed $\alpha_{1}$- and $\alpha_{2}$-antagonist phentolamine blocked the CBF response elicited by norepinephrine and epinephrine. Administration of the selective $\alpha_{2}$-antagonist idazoxan demonstrated no effect on the $\alpha$-agonist–elicited response. These results suggest that $\alpha_{2}$-receptors are not involved in the local control of CBF. Similarly, the response to epinephrine and norepinephrine was unaltered after administration of propranolol, a selective $\beta$-antagonist. Thus, only $\alpha_{1}$-adrenergic receptors may be present in the cochlear vasculature. However, it must be emphasized that with the topical route of administration, the concentration of each drug in the inner ear could not be specified. Thus, it is possible that the negative effects observed with idazoxan and propranolol, tested alone and in the interaction studies, may not be due to the absence of appropriate receptors but rather an inadequate level of drug at the receptor. This does not seem a likely explanation, given the concentration range applied to the RW and the relative size of the molecules of idazoxan and propranolol (253.6 and 295.8, respectively). Moreover, the doses of the agonist drug against which these antagonists were tested covered a range of relatively low values, so that blockage would be readily detected. Negative results may also reflect a relatively low density of these receptors, in which case their effect would be difficult to detect with any method.

Pharmacologically, greater confidence can be placed in the identification of a receptor subclass with demonstration of a consistent dose-dependent result in an antagonist–agonist interaction. In this study, when a constant high dose of the antagonist prazosin was applied, before administration of a range of doses of epinephrine or norepinephrine, a parallel rightward shift in the dose–response function was observed (Figure 4A). When topical application and systemic administration of prazosin were combined, a higher degree of blocking effect was reached, which also resulted in a further reduction of the maximum response elicited by the highest concentrations of epinephrine and norepinephrine tested (Figure 4B).

The observation of a 100% prolongation of the response to norepinephrine in the presence of the active reuptake inhibitor desmethylipramine provides a pharmacological demonstration that this transmitter must be operating on postsynaptic receptors. It also demonstrates that, for the adrenergic system of the inner ear, a reuptake by presynaptic membranes and a breakdown of the transmitter are principal mechanisms for limiting responsiveness.

Finally, a similar and selective $\alpha$-blocking effect on both the topical and systemic epinephrine-induced CBF change with the irreversible antagonist phenoxymethamphetamine was observed. Significantly, at effective antagonist levels, this drug causes little change in BP in normotensive subjects. The elimination of all topically induced epinephrine responses after antagonist administration is consistent with the finding that only adrenergic receptors are present in the cochlear vasculature. Additionally, no response could be elicited even with the addition of the $\beta$-agonist isoproterenol to the RW in these phenoxymethamphetamine preparations.
Taken as a whole, the data of this study strongly indicate that mechanisms are available in the cochlear vasculature for sympathetic control of CBF mediated by receptors, probably only $\alpha_1$-receptors. Our observations are consistent with the view that CBF is normally under some sympathetic tone, along with other regulating factors, and can be modulated.

Identification and analysis of these local adrenergic receptors were mainly made possible by the strategy of topical application. Figure 5 clearly demonstrates that CBF response is markedly different with the systemic route of administration. First, as expected with intravenous epinephrine, CBF increased with BP in a dose-dependent fashion. With topical epinephrine, CBF declined in the presence of a stable BP. Second, at higher concentrations of intravenous epinephrine (>20 mg/kg), CBF did not continue to increase with increasing BP; in fact, CBF fell, perhaps reflecting the local vasoconstriction. The data indicate that the topical method of applying drugs to the inner ear is effective. However, there are some reservations regarding this strategy. Some preparations demonstrated no response to topical application. The percentage was clearly significant, although some preparations (2% of the total population) were also insensitive to intravenous administration as well. Other studies have shown that the local response from an organ is in fact an integrated response that will depend on differences in resting tone and a variety of related mechanisms (e.g., neuropeptides, endothelium-derived relaxing factor, intracellular Ca$^{2+}$ concentration, metabolites, and other active homeostatic tissue factors). In these preparations, unlike isolated organ preparations, such factors, of course, are not so readily under the experimenters' control and must be considered in future studies of the mechanisms of CBF.

Acknowledgments

The authors wish to thank Dr. Benedict R. Lucchesi for valuable suggestions on pharmacological evaluation of adrenergic receptors, Nadine Brown for excellent technical assistance and advice, and Yvonne Beerens and Mary Stuckmeyer for their assistance in the preparation of the manuscript.

References

33. Jagadeesh G, Deth R: Different affinity states of $\alpha_1$ adrenergic receptors defined by agonists and antagonists in bovine aorta plasma membranes. J Pharmacol Exp Ther 1987;243:430–436
34. Hedlund H, Andersson K-E, Larsson B: Effect of drugs interacting with adrenergic receptors and muscarinic receptors in the


**KEY WORDS**  ·  α-receptors  ·  β-receptors  ·  laser Doppler flowmetry  ·  guinea pigs  ·  blood pressure  ·  vasoconstrictors
Influence of topically applied adrenergic agents on cochlear blood flow.
K A Ohlsén, D L Baldwin, A L Nuttall and J M Miller

doi: 10.1161/01.RES.69.2.509

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/69/2/509

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/