Sound Stimulation-Induced Vasomotor Reflex in the Central Artery of the Rabbit Ear

MICHIKO TAKEOKA, GOU UEDA, KICHIRO TAGUCHI*, RI-LI GE, KOJI TERASAWA and KATSUMI TSUCHIYA†

Research Center for Aging and Adaptation, *Department of Oto-laryngology, Shinshu University School of Medicine, Matsumoto 390, and †Department of Environmental Physiology, Nagasaki University, Nagasaki 852

TAKEOKA, M., UEDA, G., TAGUCHI, K., GE, R.-L., TERASAWA, K. and TSUCHIYA, K. Sound Stimulation-Induced Vasomotor Reflex in the Central Artery of the Rabbit Ear. Tohoku J. Exp. Med., 1996, 178 (2), 101-111 —— Effects of sound stimulation on the central artery of the rabbit ear were studied as a somato-autonomic reflex. Vasoconstriction and dilatation, caused by metronome sound stimulation, were estimated from the temperature fluctuations in the central artery of the ear, measured by a thermistor. To enhance the detection of temperature rises, moderately high background levels of arterial tone were established by exposing the tips of the ears to water at a temperature of 10°C or 5°C, prior to sound stimulation. A fall in arterial temperature due to vasoconstriction was observed immediately after the start of the 1-min sound stimulation, with a subsequent temperature rise which overshot the original basal level due to vasodilatation. A positive correlation between the ear temperature before sound stimulation and the temperature fall (p < 0.01), and a negative correlation between the ear temperature and the temperature rise (p < 0.05) were obtained. The temperature fall was blocked by phenoxybenzamine (9 mg/kg, i.p., p < 0.01). The subsequent rise was not influenced by atropine (3 mg/kg, i.p.) or phenoxybenzamine, however, it was attenuated by hexamethonium (6 mg/kg, i.p., p < 0.05). The temperature fall at the beginning of sound stimulation was related to alpha-adrenergic mechanism. The subsequent temperature rise was thought to be related to parasympathetic mechanism, excluding cholinergic mechanism. —— adrenergic vasoconstriction; non-cholinergic vasodilatation; local cooling; parasympathetic vasodilatation

It has been shown in animals that stressful stimuli such as pinching, heat and electrical stimulation may produce marked blood flow changes in the facial skin (Karita and Izumi 1992). A number of mechanisms could be underlying the possible pain-induced blood flow changes. Firstly, studies have shown that antidromic activation of primary afferent fibers may result in increased peripheral blood flow which can be determined by laser Doppler flowmetry (Blumberg and

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Address for reprints: Michiko Takeoka, Ph.D., Research Center for Aging and Adaptation, Shinshu University School of Medicine, Asahi 3-1-1, Matsumoto 390, Japan.
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Wallin 1987; Oberle et al. 1988). This axon reflex seems to be induced preferentially by activation of nociceptive C fibers, and stressful antidromic stimulation of peripheral nerves may also activate postganglionic sympathetic fibers, which explains local vasoconstriction preceding an axon reflex which in turn causes vasodilatation (Blumberg and Wallin 1987). Moreover, painful stimulation can also cause a more wide spread, centrally mediated, sympathetic vasoconstriction reflex (Blumberg and Wallin 1987). Additionally, in the facial area, a centrally mediated parasympathetic vasodilatation reflex has been described (Drummond 1993).

Besides these mechanical, thermal and electrical stimuli, auditory stimulation was also reported to elicit a somato-autonomic response. The effect of infrasonic stimulation on blood pressure (Danielsson and Landström 1985), morphological changes of the spiral vessel after rock music exposure (Okada et al. 1991), and respiration and heart rate responses to sudden noise (Anderssen et al. 1993) have been studied.

The purpose of this study was to examine the effect of auditory stimulation on the temperature of the central artery of the ear in rabbits, due to vasoreaction. Additionally, we attempted to elucidate the mechanisms underlying the temperature changes induced by sound stimulation by use of cholinergic, adrenergic and ganglionic blockers.

**Methods**

Male rabbits weighing 2.5-3.5 kg, given ad libitum water and solid food (ORC4; Oriental, Chiba) living in an experimental animal institute at 17.5±2.5°C, were used. The experiments were performed in a soundproof artificial climatic chamber (Koito Industries, Yokohama) at 25±1°C. Since the response of the central artery of the rabbit ear to local cold exposure is greatest in May, June and July (Takeoka 1990), the experiment was carried out in June. To maintain moderately high background levels of arterial tone throughout the experiment (Kalsner 1974), ears were locally exposed to cold water prior to sound stimulation. Two kinds of background level were prepared by application of water at two different temperatures, i.e., 10°C or 5°C. Eleven unanesthetized rabbits were exposed to 10°C water, and 10 rabbits to 5°C.

Animals were trained to be sedate when fixed for a 50-min period in the supine position, on two occasions, two days and one day before the experiments. During the experiment, no physical movement was elicited by sound stimulation. A thermistor (MGA III-219; Nihon Kohden, Tokyo) sensor was taped over the central artery of the right ear, at 1/3 of the distance from the tip of earlobe (Fig. 1). The diameter of the sensor was 1 mm. The resolution of the thermistor was 0.01°C and its time constant was 2 sec. The thermistor was connected to a chart recorder (D-72B; Rikendenshi, Tokyo).

Both ears, with the sensor in place in the right ear, were held in the inner
chamber of a custom-built double chamber apparatus. Water at a temperature of 10°C or 5°C was poured into the inner chamber until it reached a level 5 mm above the thermistor sensor. In order to prevent the influence of water flow, a constant temperature unit (F2-C; Haake, Tokyo) was used to circulate the water through the outer chamber only. The water temperature of the inner vessel was maintained at 10°C or 5°C throughout the experiments.

One minute of sound stimulation was applied to the animal using a metronome placed 20 cm from the ears, 30–40 min after onset of immersion of the earlobes in water of 10°C or 5°C. The heart rate was measured from ECG R-R intervals, obtained by precordial leads using needle electrodes (FD-16; Fukudadenshi, Tokyo), every 5 min throughout the experiment, and one min before and after the beginning of stimulation.

Sound stimulation was produced twice per second by a metronome. The frequency spectra of the auditory stimuli were analyzed by a Dynamic Signal Analyzer (3562A; YHP, Tokyo). The 20-msec sound of the metronome had a frequency between 2–10 KHz with peaks appearing at 4 and 8 KHz. Its level of loudness was 76±2 phon (Sound Level Meter, Rion, Tokyo), which was converted to an intensity level using the Fletcher and Munson curve (Fletcher and Munson 1933). The converted value was 76 dB (2 KHz)-86 dB (10 KHz). Preliminary experiments with stimulation periods of 30 sec for one min and three min showed approximately equivalent results. A few initial sounds appeared to elicit vasoreaction and the nervous system seemed to adapt to the stimulation after that.

The effect of atropine (muscarinic blocker) or hexamethonium (ganglionic blocker) on temperature rises caused by sound stimulation in the central artery of the ear was investigated under 5°C local exposure conditions. Atropine sulfate (3 mg/kg; Tanabe Seiyaku, Tokyo, n=6) or hexamethonium bromide (6 mg/kg; Wako Junyaku, Osaka, n=5) was injected intraperitoneally five min before exposure of the ears to cold, and sound stimulation was applied under the same
conditions as mentioned above.

The effect of phenoxybenzamine (alpha-adrenergic blocker) on temperature falls in the central artery of the ear, caused by sound stimulation, was investigated under 10°C local exposure conditions in the ears of 6 rabbits. Phenoxybenzamine hydrochloride (9 mg/kg; Nakarai Kagaku, Kyoto) was injected intraperitoneally 5 min before exposure of the ears to the cold water, and sound stimulation was applied under the same conditions as mentioned above.

Statistics. The data were expressed as mean \pm s.d. Student’s non-paired t-test was used for statistical analysis. Differences were considered significant at \( p < 0.05 \).

Results

Temperature changes in the central artery of the ear in response to sound stimulation

The method of measuring the temperature fall due to vasoconstriction and the temperature rise due to vasodilatation in response to stimulation is as follows: the level of a temperature fall is the difference between the temperature at the onset of the fall and the following minimum, and that of a temperature rise is the

![Graph showing temperature changes in the central artery of the ear](image)

Fig. 2. Reactions of central artery of the ear caused by sound stimulation under 10°C local exposure conditions. A fall in the temperature due to vasoconstriction occurred immediately following the commencement of sound stimulation, which was followed by a temperature rise due to vasodilatation (upper). The reaction of the central artery of the ear caused by sound stimulation under 5°C local exposure conditions (lower).
difference between the temperature at the onset and the maximum temperature (Fig. 2). The typical response of the central artery of the right ear to 1-min sound stimulation under 10°C local exposure conditions is shown in Fig. 2 (upper). A temperature fall was observed immediately after the beginning of sound stimulation in all 11 arteries. Furthermore, an overshooting temperature rise occurred after the initial fall in 9 out of the 11 arteries. A temperature fall without subsequent temperature rise was observed in the remaining 2 arteries.

The typical response of the central artery of the ear to sound stimulation under 5°C local exposure conditions is shown in Fig. 2 (lower). Both a fall and a rise in temperature were observed in 8 out of the 10 arteries, whereas only a rise was observed in 2 arteries.

*Changes in temperature due to vasoreaction in the central artery of the ear*

A positive correlation between the ear temperature before sound stimulation and the temperature fall evoked by sound stimulation \( (p < 0.01) \), and a negative correlation between the ear temperature and temperature rise \( (p < 0.05) \) were obtained (Fig. 3).

The temperature fall was \( 1.59 \pm 0.65°C \) \( (n = 11) \), and the subsequent temperature rise was \( 0.88 \pm 0.59°C \) in the ears which underwent 10°C cooling. The arterial temperature before stimulation was \( 15.88 \pm 2.58°C \). In the ears which underwent 5°C cooling, the temperature fall was \( 0.39 \pm 0.29°C \) \( (n = 10; p < 0.01 \) vs. 10°C.

![Graph showing temperature changes](image)

*Fig. 3.* Correlations of ear arterial temperatures before stimulation with temperature fall \( (Y = -4.012 + 0.518X, r = 0.785, p < 0.01) \), and temperature rise \( (Y = 10.082 - 0.423X, r = 0.492, p < 0.05) \) caused by sound stimulation. 10°C (○), 5°C (●).
exposure conditions; 19 d.f.) and the subsequent temperature rise was $2.04 \pm 0.99 \text{°C}$ ($p < 0.01$ vs. 10°C exposure conditions; 19 d.f.). The arterial temperature before stimulation was $11.22 \pm 2.83 \text{°C}$ (Fig. 4).

**Heart rate**

In the rabbits analyzed under both 10°C and 5°C exposure conditions, no difference was seen in their heart rates between 10 min before and one min after immersion of the earlobe into water. The heart rate after the beginning of sound stimulation was slightly higher than the heart rate one min before stimulation. Cooling and sound stimulation had no significant effect on heart rate.

**Atropine**

The effect of atropine or hexamethonium on temperature rises in the central artery of the ear caused by sound stimulation was investigated under 5°C local cooling conditions, because the rise was most pronounced in these conditions. The temperature fall in the artery immediately after the beginning of sound stimulation was $0.52 \pm 0.35 \text{°C}$ ($n=6$) and the subsequent temperature rise was $1.78 \pm 1.09 \text{°C}$ in atropine-pretreated rabbits. The arterial temperature before stimulation was $12.05 \pm 1.94 \text{°C}$. No significant difference was seen between these values and the control values.

The heart rates 5 min before atropine injection, during the ear cooling process before sound stimulation, and one min after the beginning of stimulation were $269.4 \pm 15.4$ beats/min, $289.4 \pm 12.2$ beats/min ($p < 0.05$ vs. 5 min before atropine injection), and $291.5 \pm 23.1$ beats/min respectively. The effect of atropine on heart rates was significant, whereas cooling and sound stimulation had no effect on
Fig. 5. Effect of hexamethonium on temperature fall (◻) and rise (□) in the central artery of the ear caused by sound stimulation under 5°C exposure conditions (mean ± s.d.).

*p < 0.05; n.s., not significant.

Heart rate.

Hexamethonium

The temperature fall in the artery immediately after beginning of sound stimulation was 0.33 ± 0.25°C, the subsequent temperature rise was 1.73 ± 0.38°C, and the arterial temperature before stimulation was 12.56 ± 1.76°C in rabbits under 5°C exposure conditions (control, n = 5, Fig. 5). Correspondingly, in hexamethonium-pretreated rabbits (n = 5), the temperatures were 0.20 ± 0.21°C, 0.84 ± 0.64°C (p < 0.05 vs. control), and 13.33 ± 1.91°C, respectively.

The heart rates during the ear cooling process before sound stimulation, and

Fig. 6. Effect of phenoxybenzamine on temperature fall (◻) and rise (□) in the central artery of the ear caused by sound stimulation under 10°C exposure conditions (mean ± s.d.).

**p < 0.01; n.s., not significant.
one min after beginning of stimulation were higher than the heart rates 5 min before hexamethonium injection, however, no significant differences were seen among them. Cooling and sound stimulation had no effect on heart rate.

**Phenoxybenzamine**

The effect of phenoxybenzamine on temperature falls in the central artery of the ear caused by sound stimulation was investigated under 10°C local exposure conditions, since the fall was most pronounced in these conditions. The temperature fall in the artery immediately after the beginning of sound stimulation was $0.05 \pm 0.08^\circ$C ($n=6$), and the subsequent temperature rise was $0.25 \pm 0.47^\circ$C in phenoxybenzamine-pretreated rabbits. The arterial temperature before stimulation was $16.23 \pm 2.03^\circ$C. The inhibitory effects of phenoxybenzamine, on temperature falls evoked by sound stimulation were significant ($p < 0.01$, Fig. 6).

No significant differences were seen among the heart rates 5 min before phenoxybenzamine injection, during the ear cooling process before sound stimulation, and one min after beginning of stimulation. Cooling and sound stimulation had no effect on heart rate.

**Discussion**

An earlier study showed that a buzzer sound caused vasoconstriction in normal adults, as assessed using a digital plethysmograph, whereas no changes were observed in deaf subjects (Winsor et al. 1956). Some later studies have reported the vasomotor response to sound stimulation (Mojdehi et al. 1980; Sarb and Weiler 1983), however these have referred only to vasoconstriction. This may have been due to ignorance of maintaining a moderately high background level of arterial tone. In our experiments, temperature rises, temperature falls, or both occurred in the central arteries of the ears of rabbits in response to sound stimulation under earlobe cooling condition.

There are two pathways involved in the medullary reticular formation: a fast (neospinothalamic) system and a slow (paleothalamic) system. Since the hypothalamus is involved in the paleothalamic system, the auditory autonomic response may be caused by the latter system. Descending from vasomotor centers, the neural impulse reaches the sympathetic system, which conveys the impulse to the smooth muscle cells of the terminal arteries, arterioles, precapillary sphincters, and arteriovenous shunts (Mojdehi et al. 1980). The smooth muscle cells in the precapillary bed alter the tone of the vessel, producing either an increase or decrease in blood flow (Mojdehi et al. 1980).

**Thermistor**

We chose a noninvasive thermistor to evaluate vascular responses because even a slight needle puncture markedly alters arterial blood flow (Takeoka and Ueda 1988). Although the thermistor is influenced by ambient temperature, the
temperature of the finger skin in 0°C-cold water measured with a thermistor was significantly correlated to blood flow of the finger (Takeoka et al. 1995). Therefore, temperature obtained with the thermistor is considered to be reliable.

**Vasoconstriction**

According to Bini et al. (1980), sudden noise elicits a sympathetic reflex volley in the peroneal nerve skin fascicle. In our study, sound stimulation had no effect on the central arteries of rabbit ears pretreated with phenoxybenzamine: i.e., the temperature fall caused by sound stimulation is caused by an alpha-adrenergic mechanism.

**Vasodilatation**

To investigate the possibility of vasodilatory innervation, it is necessary to maintain a moderately high level of arterial tone. This was accomplished by Kalsner (1974) by treatment with norepinephrine, however, in our experiments, cold was applied locally.

Cholinergic innervation is thought to be responsible for the vasodilatation occurring in the canine femoral artery (Angus et al. 1983), and rabbit ear vessels (Owen and Bevan 1985). Acetylcholinesterase is present in the nerve bundles of rabbit ear vessels (Iijima and Tagawa 1976). However, vasodilatation caused by electrical stimulation is not blocked by atropine (Kalsner 1974; Bell et al. 1985). Since cold-induced vasodilatation is caused by a cessation of transmitter release from adrenergic nerve ending (Gardner and Webb 1986), reduction of sympathetic nerve innervation is thought to decrease tone in the artery, i.e. vasodilatation.

However, in the facial area a parasympathetic vasodilatory reflex was reported (Izumi and Karita 1992; Karita and Izumi 1992; Drummond 1993; Kemppainen et al. 1994). In our experiments, temperature rises in the central artery of the ear, caused by sound stimulation, were resistant to blockage by atropine (antimuscarinic) and phenoxybenzamine (antidrnergic; Fig. 6), but they were attenuated by hexamethonium (ganglionic blocker; Fig. 5), suggesting that the temperature rises in the central artery of the ear caused by sound stimulation were effected via parasympathetic neurons that are not cholinergic.

**Influence of local cooling temperature**

In this study, large temperature falls and small temperature rises occurred in the central arteries of rabbit ears by sound stimulation in rabbits under 10°C cooling conditions, and small temperature falls and large temperature rises occurred under 5°C cooling conditions. A positive correlation between the ear temperature before stimulation and the temperature fall, and a negative correlation between the ear temperature and the temperature rise, were obtained. In Blumberg's study (1987) with laser-doppler flowmetry and photo-electrical pulse plethysmography, warming diminished vasodilatation evoked by electrical stimulation
of the peroneal nerve in the skin, whereas cooling enhanced it. According to Oberle et al. (1988), intraneural electrical stimulation was accompanied by changes in blood flow in the skin of the foot; warm (>30°C) subjects responded with cutaneous vasoconstriction, whereas cold (<25°C) subjects responded with vasodilatation. Under cold conditions arterio-venous shunts may have already been closed and only vasodilatation due to opening the shunts would then be possible to detect. Another explanation is that arteries are constricted under the cold conditions and relaxation of arterial smooth muscle would then be detectable.

Heart rate

According to Sato et al. (1976), the response to nociceptive dermal stimulation of the rat was an increase in the heart rate at its rectal temperature of 38.0–38.9°C, but a decrease at 36.0–36.9°C. Thus, the reason why we observed no significant increase in heart rate with sound stimulation is thought to be due to the low rectal temperature induced by immobilizing the rabbits for around 40 min and then local cooling of the ears.

As far as relationships between exposure to noise and mean blood pressures, no difference was reported between men with hearing defect and men with normal hearing (Drettner et al. 1975; Takala et al. 1977). On the other hand, low frequency noise was shown to affect the blood pressure mainly by increasing the diastolic pressure without increasing the pulse rate (Danielsson and Landström 1985). Since a needle puncture markedly alters the arterial blood flow (Takeoka and Ueda 1988), blood pressure was not measured in our experiment.

In conclusion, most central arteries of rabbit ears showed temperature falls followed by temperature rises in response to sound stimulation. It is still unclear whether the mechanisms underlying vasoreactions are similar to those for mechanical, thermal or electrical stimulation. However, the temperature falls which occurred immediately after beginning of sound stimulation were related to alpha-adrenergic mechanism. The temperature rise which followed this may be related to parasympathetic mechanism, excluding cholinergic mechanism.

References

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