

An Approach to the Evaluation of Sympathomimetic Amines by Measuring the Decrease of Ear Temperature in Rabbits and Dogs¹⁾

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(Received February 5, 1973)

An attempt was made to quantitatively evaluate the effect of sympathomimetic amines by measuring the decrease in ear temperature, which reflects the constriction of the peripheral vessels, in intact rabbits and dogs.

In rabbits, the decrease in ear temperature was proportional to the increasing dose of epinephrine, norepinephrine or ethylphenylephrine when the drugs were given intravenously. The linear regression between the logarithmic dose and the area under time-decreasing curve was highly significant for each drug. The action of *l*-epinephrine was approximately two times more effective than that of *dl*-norepinephrine, and the effect of ethylphenylephrine persisted longer than that of the others. Little decrease in ear temperature was observed after intravenous injection of isoproterenol. The ear temperature lowering action of norepinephrine and ethylphenylephrine was inhibited by the pretreatment with phenoxybenzamine.

In rabbits and dogs, when ethylphenylephrine was orally given, the linear regression between the logarithmic dose and the area under time-decreasing curve was also highly significant.

The present results indicate that the determination of ear temperature is useful for the evaluation of potencies of preparations containing the vasoconstrictors tested.

Sympathomimetic amines (vasoconstrictors) have been known to decrease the skin temperature as a result of constriction of the peripheral vessels. Therefore, the measurement of skin temperature has been used for a clinical evaluation of adrenergic drugs.³⁾ However, little attempt has been made to quantitating this action in clinical evaluation or in animal experiments. The present work was planned to evaluate quantitatively the intensity and duration of the temperature lowering effect of sympathomimetic amines by measuring the decrease of ear temperature continuously in rabbits and dogs.

Material and Method

Epinephrine (Adrenalin® containing 1 mg of *l*-epinephrine per ml) and norepinephrine (Noradrelin® containing 1 mg of *dl*-norepinephrine per ml) were diluted with physiological saline immediately before use. Racemic isoproterenol sulfate and phenoxybenzamine hydrochloride was dissolved in physiological saline. Racemic ethylphenylephrine hydrochloride was dissolved in physiological saline for injection and filled in gelatin capsule for oral administration. Thermister thermometer (Natume Seisakusho, Type NS-3P), which connected the rectal-applicator of polyethylene for rats, was used.

Healthy male rabbits weighing 2.2–2.8 kg were used under the condition of restraining their neck. The applicator of thermometer was fixed on the skin surface of the inside of an ear with the adhesive plaster. The control ear temperature of each animal was measured 60, 30 min and immediately before the administration of drugs. The rabbits, whose ear temperature varied within 1° during 60 min period, were used. Epinephrine (25, 50, and 100 µg), norepinephrine (50, 100, and 200 µg), ethylphenylephrine (250, 500, and 1000 µg), isoproterenol (20 and 200 µg) or 1 ml of physiological saline was injected in the ear vein. Five minutes after intravenous injection of 2.5 mg of phenoxybenzamine, 100 µg of norepinephrine or 500 µg of

1) A part of this report was presented at the 44th Regional Meeting of Kanto Area of the Japanese Pharmacological Society, Yokohama, June 1971.

2) Location: *Hiromachi 1-chome, Shinagawa-ku, Tokyo.*

3) B. Calesnick, "Animal and Clinical Pharmacologic Techniques in Drug Evaluation," ed. by J. H. Nodine and P. E. Siegler, Year Book Medical Publisher Inc., Chicago, 1964, p. 111.

ethylphenylephrine was administered intravenously. Ethylphenylephrine (30, 60, and 120 mg) was orally given with 100 ml of water in the rabbits fasted for about 18 hr. The ear temperature was measured at intervals of 1 min during the first 15 min and at 20, 25, 30, 40, 50, and 60 min after the intravenous injection of drugs. In the case of oral administration of drugs the ear temperature was measured at intervals of 30 min over 6 hr.

Healthy 6 mongrel dogs weighing 8–13 kg were used under the condition of no restraint after fasting for about 18 hr. The applicator of thermometer was enveloped in the inside of an ear and the temperature was recorded 2 min later. The dogs were used for each test after having verified that the difference between the temperatures at 30 min before and at just before administration of drugs was within 0.5° . The changes of ear temperature in 6 untreated dogs were recorded over 8 hr. These dogs had a rest period of one week and were given crossedly each dose of 30, 60, or 120 mg of ethylphenylephrine at weekly intervals. After administration of the drug, the ear temperature was measured at every 30 min over 8 hr period.

All experiments were carried out in a room keeping temperature at $23 \pm 1^{\circ}$ and relative humidity 50–70%. The ear temperature measured after administration of drugs was compared with that obtained immediately before administration. The changes in ear temperature were plotted against the time after administration of the drugs and the area under changing curve was measured with planimeter.

Result

Changes in Ear Temperature after Intravenous Injection of Sympathomimetic Amines in Rabbits

The ear temperature significantly decreased after intravenous injection of epinephrine, norepinephrine or ethylphenylephrine (Fig. 1–3). The maximum decrease was about 4°

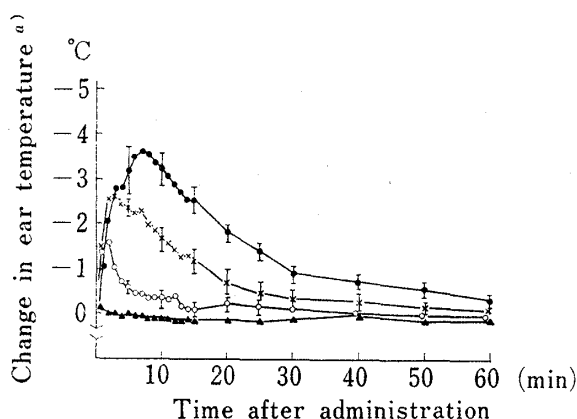


Fig. 1. Mean Changes in Ear Temperature after Intravenous Injection of Epinephrine in Rabbits

—○—: 25 μ g/rabbit ($n=5$)
 —x—: 50 μ g/rabbit ($n=5$)
 —●—: 100 μ g/rabbit ($n=5$)
 —▲—: physiological saline ($n=5$)
 a) Vertical lines indicate the standard error of the mean.

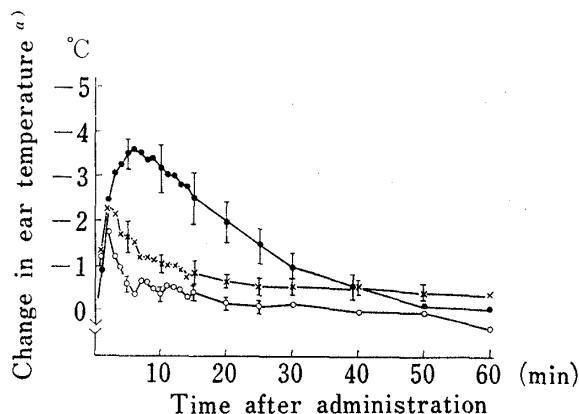


Fig. 2. Mean Changes in Ear Temperature after Intravenous Injection of Norepinephrine in Rabbits

—○—: 50 μ g/rabbit ($n=5$)
 —x—: 100 μ g/rabbit ($n=5$)
 —●—: 200 μ g/rabbit ($n=5$)
 a) Vertical lines indicate the standard error of the mean.

for 100 μ g of epinephrine and 200 μ g of norepinephrine, and 6° for 1000 μ g of ethylphenylephrine. The degree and duration of a decrease in ear temperature was larger with increasing the dose in these three drugs. The relationships between the logarithmic doses and the areas under decreasing curve in ear temperature are presented in Fig. 4. A highly significant linear regression was obtained in each drug.

The changes in ear temperature after intravenous injection of isoproterenol is shown in Fig. 5. A significant decrease, about 2° at the peak, was demonstrated in a dose of 200 μ g of isoproterenol. The lowered temperature by isoproterenol, however, returned to the control level within 10 min. Little change was observed in a dose of 20 μ g of isoproterenol.

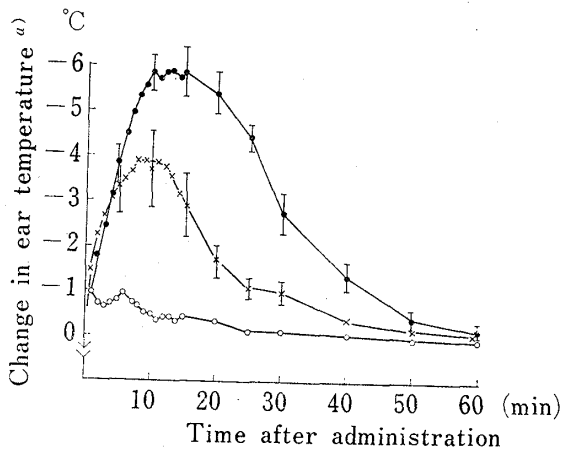


Fig. 3. Mean Changes in Ear Temperature after Intravenous Injection of Ethylphenylephrine in Rabbits

—○—: 250 µg/rabbit (n=5)
 —×—: 500 µg/rabbit (n=5)
 —●—: 1000 µg/rabbit (n=5)
 a) Vertical lines indicate the standard error of the mean.

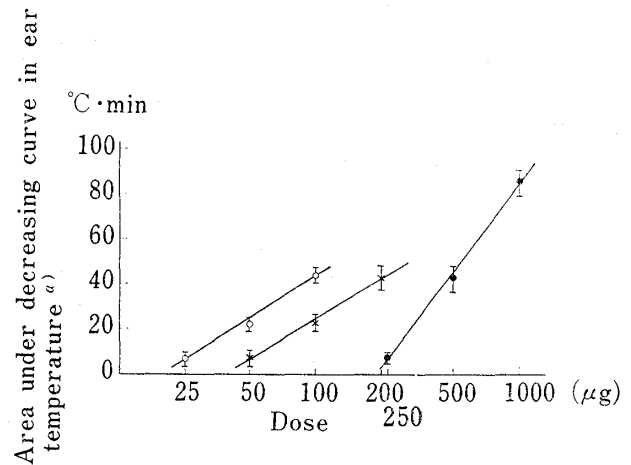


Fig. 4. Relationship between the Doses of Sympathomimetic Amines and the Areas under Decreasing Curve in Ear Temperature as shown in Fig. 1, 2, and 3

—○—: epinephrine
 —×—: norepinephrine
 —●—: ethylphenylephrine
 a) Vertical lines indicate the standard error of the mean.

Changes in Ear Temperature after Oral Administration of Ethylphenylephrine in Rabbits and Dogs

Figure 6 shows the changes in ear temperature in rabbits after oral administration of three doses of ethylphenylephrine. Ethylphenylephrine significantly lowered the ear temperature in all doses tested. The maximum decrease, about 10°, was observed in the rabbits given 120 mg of ethylphenylephrine. Much larger intensity and longer duration of the decrease in ear temperature were demonstrated with increasing the dose. The linear regression

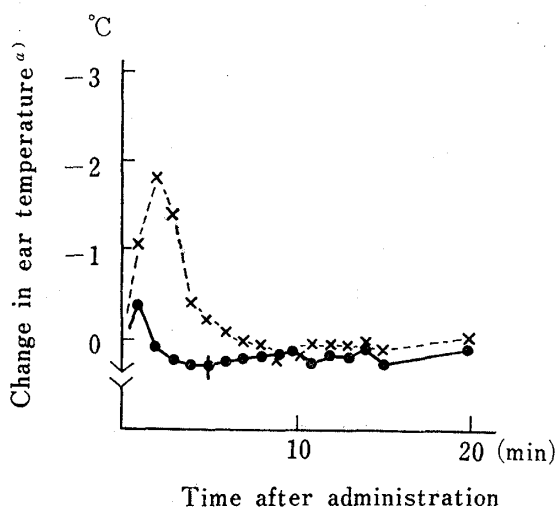


Fig. 5. Mean Changes in Ear Temperature after Intravenous Injection of Isoproterenol in Rabbits

—●—: 20 µg/rabbit (n=3)
 —×—: 200 µg/rabbit (n=3)
 a) Vertical lines indicate the standard error of the mean.

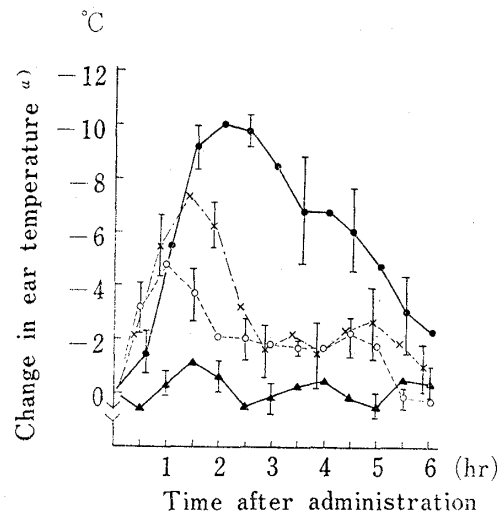


Fig. 6. Mean Changes in Ear Temperature after Oral Administration of Ethylphenylephrine in Rabbits

—○—: 30 mg/rabbit (n=5)
 —×—: 60 mg/rabbit (n=5)
 —●—: 120 mg/rabbit (n=5)
 —▲—: untreated (n=5)
 a) Vertical lines indicate the standard error of the mean.

between logarithmic doses of ethylphenylephrine and the area under decreasing curves was highly significant (Fig. 7).

In dogs, a significant decrease in ear temperature was also demonstrated after oral administration of ethylphenylephrine (Fig. 8). The maximum decrease, about 3.5°, was shown

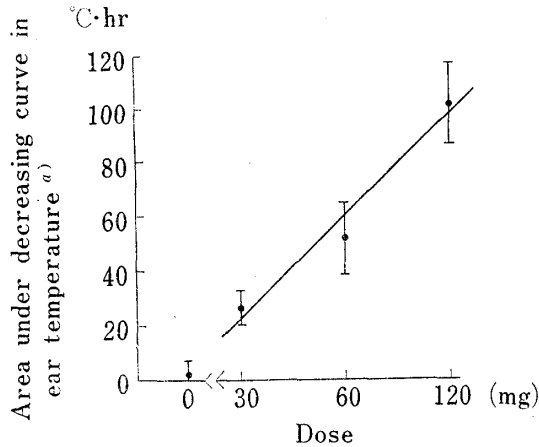


Fig. 7. Relationship between Oral Dose of Ethylphenylephrine and the Area under Decreasing Curve in Ear Temperature as shown in Fig. 6

a) Vertical lines indicate the standard error of the mean.

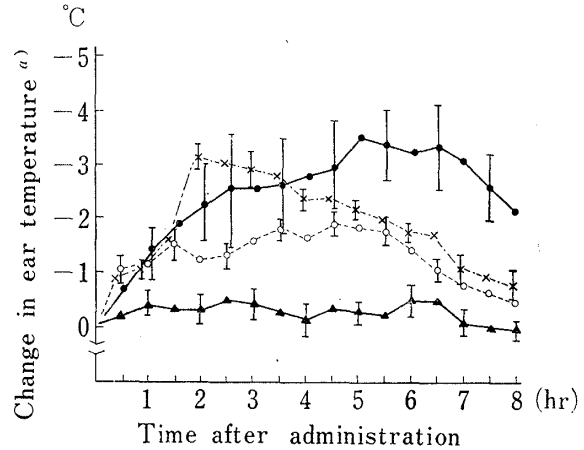


Fig. 8. Mean Changes in Ear Temperature after Oral Administration of Ethylphenylephrine in Dogs

—○—: 30 mg/dog (n=6)
 —×—: 60 mg/dog (n=6)
 —●—: 120 mg/dog (n=6)
 —▲—: untreated (n=6)

a) Vertical lines indicate the standard error of the mean.

in a dose of 60 mg as well as 120 mg of ethylphenylephrine. However, the decrease in ear temperature reached a maximum 2 hr after administration of 60 mg of ethylphenylephrine, whereas the ear temperature continued to decrease till 5 hr after administration of 120 mg of ethylphenylephrine. The linear regression between the logarithmic doses of ethylphenylephrine and the area under decreasing curves in ear temperature was highly significant (Fig. 9).

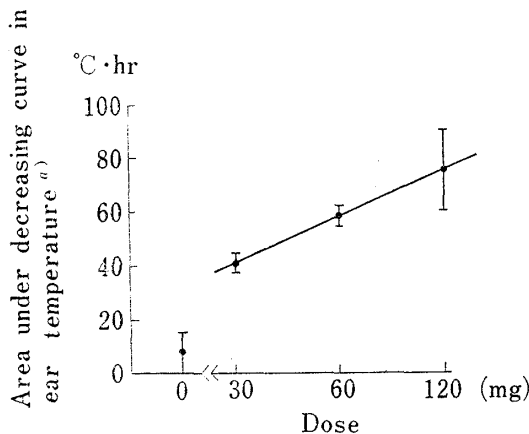


Fig. 9. Relationship between Oral Dose of Ethylphenylephrine and the Area under Decreasing Curve in Ear Temperature as shown in Fig. 8.

a) Vertical lines indicate the standard error of the mean.

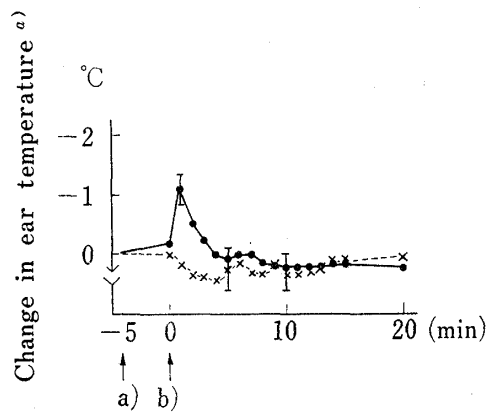


Fig. 10. Mean Changes in Ear Temperature after Intravenous Injection of Norepinephrine and Ethylphenylephrine in Rabbits pretreated with Phenoxybenzamine

a) phenoxybenzamine 2.5 mg *i.v.*
 b) —●—: norepinephrine 100 µg *i.v.* (n=5)
 ---×---: ethylphenylephrine 500 µg *i.v.* (n=3)

a) vertical lines indicate the standard error of the mean.

Influence of the Pretreatment with Phenoxybenzamine on the Ear Temperature Lowering Effect of Norepinephrine and Ethylphenylephrine

Pretreatment with 2.5 mg of phenoxybenzamine abolished the ear temperature lowering effect of norepinephrine or ethylphenylephrine. As shown in Fig. 10, a little decrease in ear temperature was observed only at the early stage after intravenous injection of 100 μ g of norepinephrine and no decrease was shown even after administration of 500 μ g of ethylphenylephrine.

Discussion

Whelan⁴⁾ has reviewed the effects of adrenergic drugs on the systemic circulation; the change of blood flow in the skin circulation was described but the change in skin temperature was not mentioned. Calensnick³⁾ has reported the techniques for measurement of skin temperature in clinical evaluation of adrenergic drugs, but he has not attempted a quantitative analysis. In the present study, the decrease in ear temperature, which reflected the constriction of the peripheral vessels, was measured to evaluate the sympathomimetic amines quantitatively.

The control ear temperature measured immediately before administration of drugs was $37.6 \pm 0.10^\circ$ ⁵⁾ in 88 rabbits and $37.4 \pm 0.09^\circ$ ⁵⁾ for 24 determinations in 6 dogs, respectively. The areas under decreasing curves in ear temperature after administration of the drugs were proportional to the intravenous doses of epinephrine or norepinephrine and to the intravenous or oral doses of ethylphenylephrine. These results indicate that the measurement of the decrease in ear temperature can be applied to the quantitative evaluation of potencies in preparations containing these sympathomimetic amines.

Epinephrine is 2 to 10 times effective in constricting the skin vessels than norepinephrine.⁶⁾ In the present experiment, epinephrine was approximately two times effective than that of norepinephrine in rabbits (Fig. 4). It indicates that *l*-norepinephrine and *l*-epinephrine is nearly equally effective in lowering the ear temperature, since norepinephrine used in the study is the racemate. The dose-response curve for ethylphenylephrine was not parallel to that of epinephrine and norepinephrine. The effect of ethylphenylephrine lasted longer than other two drugs when injected intravenously. In addition, ethylphenylephrine given orally to rabbits and dogs caused also the decrease of ear temperature. This might be due to higher stability of ethylphenylephrine in the body. Danneberg, *et al.*⁷⁾ has reported that ethylphenylephrine was stable in gastrointestinal fluid and metabolized slightly in *in vitro* experiments.

In the present experiment, isoproterenol, having β -adrenergic activity, caused a minimal decrease in ear temperature as compared with that of epinephrine or of norepinephrine and did not increase markedly the ear temperature; thus the effect of isoproterenol on ear temperature was equivocal. There are reports showing that isoproterenol causes a vasoconstriction⁸⁾ and that it causes a vasodilatation in small dose and a vasoconstriction in large dose,⁹⁾ in *in vitro* experiments using isolated ear of rabbits. The difference between these observations and the result obtained in the present experiment appears to depend upon the difference of the dose used and the experimental material; the ear of intact animal and the isolated ear.

4) R. F. Whelan, "Physiological Pharmacology," Vol. IV, ed. by W. S. Root and F. G. Hofmann, Academic Press, New York, 1967, p. 29.

5) The mean \pm S.E.

6) B. Levy and R.P. Aplquist, "Drill's Pharmacology in Medicine," 3rd ed., ed. by J.R. DiPalma, McGraw-Hill Book Co., New York, 1965, p. 463.

7) P. Danneberg, E. Hasse, R. Hahn, and R. Stalb, *Arzneim-Forsch.*, **15**, 207 (1965).

8) W.S. Gay, M.J. Rand, and J. Wilson, *J. Pharm. Pharmac.*, **19**, 468 (1967).

9) M.D. Day and H.V.R. Dixon, *J. Pharm. Pharmac.*, **23**, 98 (1971).

The decrease in ear temperature by norepinephrine or ethylphenylephrine was markedly inhibited by the pretreatment of phenoxybenzamine. These results suggest that the ear temperature lowering effect of norepinephrine or ethylphenylephrine is based upon the α -adrenergic activity of sympathomimetic amines.

Acknowledgement The authors express their gratitude to Dr. Y. Aizawa, Professor of Tokyo College of Pharmacy, for his guidance during this work, and also to Dr. H. Negoro, Director of this Laboratories, for his encouragement and permission for publication of this work. Thanks are given to Mr. T. Yoshizaki for his assistance in the experimental works.