PHARMACOLOGICAL STUDIES ON THE VASOMOTOR CENTER WITH EMPHASIS ON THE VASODILATOR NERVE

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There has been no sufficient investigation about the physiology of the vasomoter nerve, especially about the vasodilator nerve. Folkow[1) 2)] has illustrated that vasomotion is controlled only by sympathetic. Stricker[3)] stated that the vasodilator nerve was contained in the dorsal root fibres. Bayliss[4) 5)] observed that the stimulation of the peripheral end of a cut sensory nerve resulted, in certain animals, in the vasodilatation in the area of skin supplied by the nerve and he investigated that this phenomenon resulted from the antidromic impulse conduction of sensory nerve. Langley[6) 7)] dinied the existence of autonomic nerve in the dorsal root, and he also observed the vasodilatation with antidromic impulse conduction of sensory nerve. Since then many investigators[8) 9) 10) 11) 12) 13)] have studied on the same antidromic impulse conduction. Holton and Perry[14) 15) 16)] also observed this phenomenon and investigated on its transmitter. On the other hand, Kuré *et al.*[17)] have reported the existence of not only sensory nerve fibres but parasympathetic fibres in the dorsal root of cervical and thoracicolumbar cord. Umemoto[18)] has demonstrated the existence of vasodilator nerve in the rabbit's ear by using a method established by Kanda *et al.*[19)] In the present investigation, the existence of active vasodilator nerve was observed in the dorsal root fibres by means of a new experimental method, and furthermore the possibility that the nerve is parasympathetic and cholinergic was proved.

METHODS

The rabbits used in these experiments were large albinos. The right external ear was sympathetically denervated by removal of the ipsilateral stellate and superior cervical ganglia at least 7 days before the experiments. And the peripheral nerve fibres of sympathetic were degenerated completely. Thus, the sympathetic which is generally recognized as a main regulator of blood vessels was removed from a rabbit's ear of one side, and the vasomotion of the sympathectomized side was compared with that of the other side

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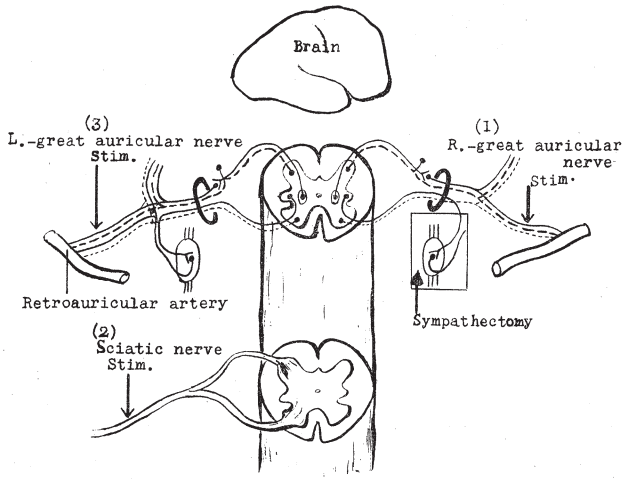

FIG. 1. Schematic drawing of the experiments 1, 2 and 3.

(intact side). Right great auricular nerve, sciatic nerve and left great auricular nerve were then stimulated respectively as shown in Fig. 1.

And in each case, the vasomotions of bilateral retroauricular arteries were recorded on oscilograph using Reflexion Photoelectric Plethysmograph (RPPG). The same recording method was used in the severing experiment of dorsal roots of right-II and III cervical nerves (r-C_2 and C_3) and in the experiment using eserine. For stimulation of the nerve fibre, an electronic stimulator was used. An unipolar saline electrode was adopted to avoid the damage of the nerve fibre due to the repeated stimulation. A saline electrode was placed on the nerve fibre and the indifferent electrode (anode) was inserted in rectum. Stimulation was done for 30 sec. with repeated square waves of strength 30 V, period 500 msec. and duration 5 msec. (the most effective strength for the observation of vasomotion with unipolar saline electrode is 30 V[14]) RPPG is an improved model by Takagi's physiological laboratory[20] based on the pri-

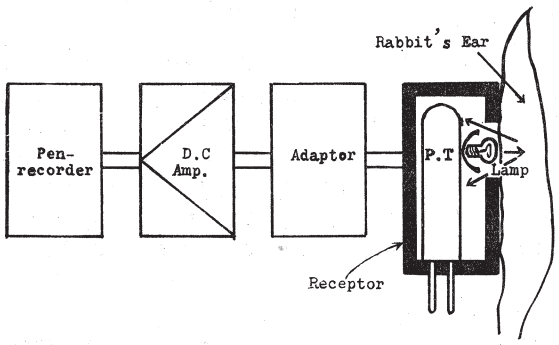

FIG. 2. Diagram of recording apparatus.

nciple of Hertzman.[21] A simplified scheme of the principle is shown in Fig. 2.

The light beam from a tiny hole of a receptor is reflected on tissue, and a phototube receives the reflected light and changes it into electric current. The current enters into D-C amplifier connected with pen-recorder through an adaptor. In this experiment, the tiny hole of the receptor was placed accurately on retroauricular artery (the most adequate pressure for attaching of the receptor to tissue is 10 mm Hg[22]). The pen-recorder was set to draw an upstroke curve on the paper when blood vessel dilated and phototube accepted less reflecting light. These experiments were done in a dark room under the room temperature not above 24.0 C.

RESULTS

1) Stimulation of the right great auricular nerve (sympathectomized side)

In about 10-15 min. from the start of recording, the vasomotion became almost stable, without the natural variation of blood vessel. When right great auricular nerve (sympathectomized nerve) was stimulated, the dilatation of bilateral retroauricular arteries was observed as shown in Fig. 3.

Vasodilatation was induced in about 90 per cent of the cases on the right side and in about 80 per cent on the left side. In most cases, this vasodilatation started within 1-2 min. after the stimulation and reached a peak in 3-5 min. and recovered to the original level within about 10 min. But in a few cases, the vasodilatation of right retroauricular artery was continued for 30 min. or more after the stimulation.

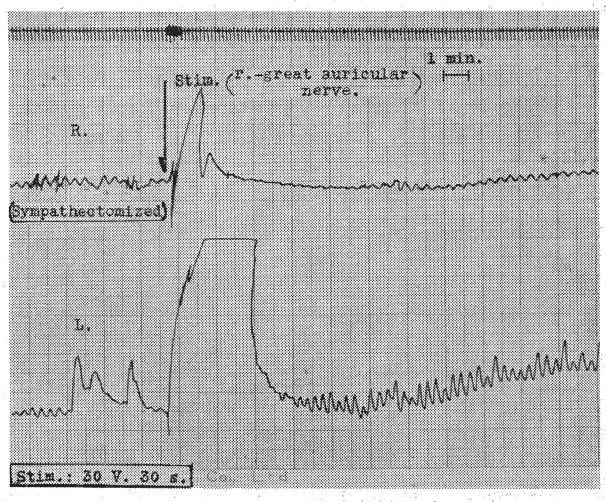

FIG. 3. The vasomotion of bilateral retroauricular arteries when the right great auricular nerve (sympathectomized) was stimulated.

2) Stimulation of sciatic nerve

When right sciatic nerve was stimulated, the vasomotion of retroauricular

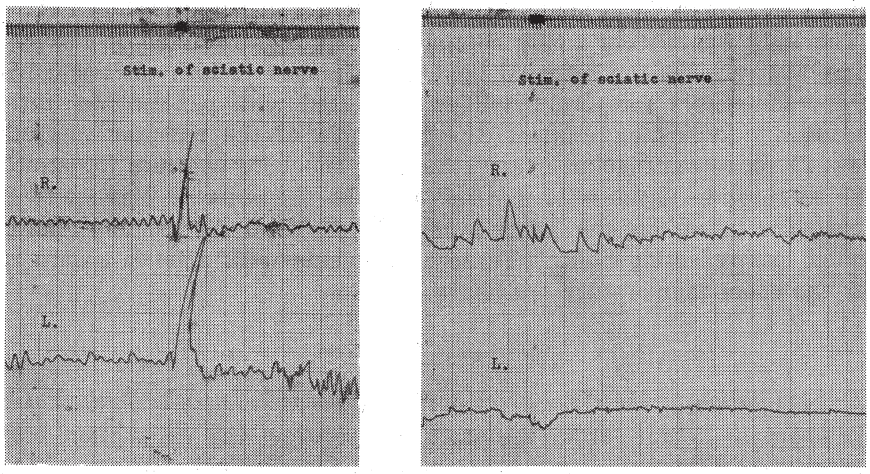

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FIG. 4. The vasomotion of bilateral retroauricular arteries when the right sciatic nerve was stimulated.

artery showed either slight dilatation or no change in both sides, as shown in Fig. 4 a and 4 b.

3) *Stimulation of left great auricular nerve (intact nerve)*

In the experiment 1, the vasodilatation of bilateral retroauricular arteries was observed with the stimulation of right great auricular nerve. It is probable that the stimulation impulse might reach the opposite side via reflex path. Therefore, in this experiment, the vasomotion of right retroauricular artery (sympathectomized side) was observed when left great auricular nerve (intact nerve) was stimulated. As shown in Fig. 5 a and 5 b, the dilatation

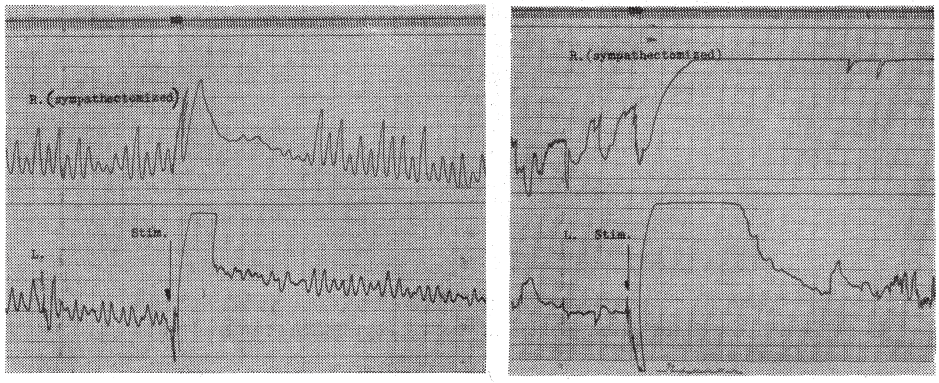

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FIG. 5. The vasomotion of bilateral retroauricular arteries when the left great auricular nerve (intact nerve) was stimulated.

of bilateral retroauricular arteries occurred, but there was great difference in occurrences of the phenomenon between the right and the left side.

Namely, left retroauricular artery was dilated in almost all cases, whereas right retroauricular artery was dilated in only 40 per cent of experiments. In one of the cases as shown in Fig. 5 b, the dilatation of right retroauricular artery continued for 30 min. or more by the stimulation of left great auricular nerve.

4) *Severing of dorsal roots of right-C_2 and C_3.*

This experiment was performed in the cases in which bilateral dilatation of the retroauricular arteries occured during the experiment 3. The scheme of this experiment is shown in Fig. 6.

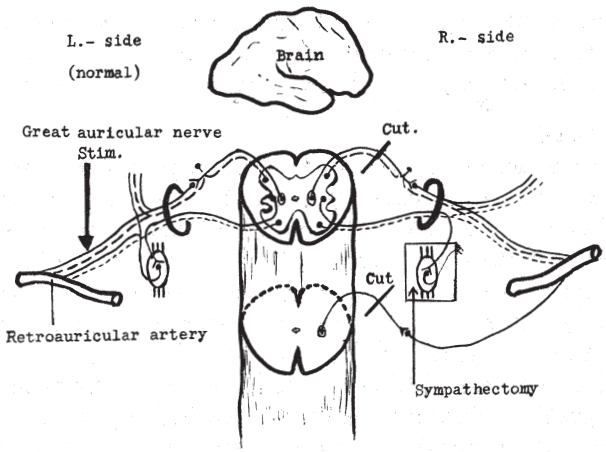

FIG. 6. Schematic drawing of the experiment 4.

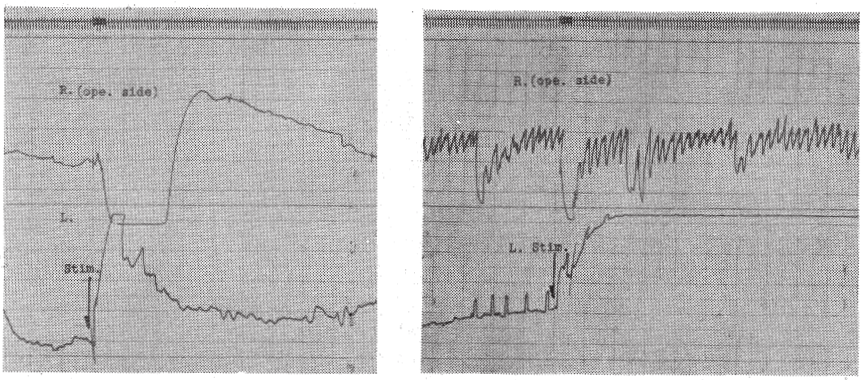

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FIG. 7. The vasomotion of bilateral retroauricular arteries with the stimulation of the left great auricular nerve (intact nerve) after severing of dorsal roots of the right-C_2 and C_3.

The rabbit sympathectomized on right external ear was fixed in ventral position, and the spinal canal was opened from back, and then dorsal roots of right C_2 and C_3 was cut. After this operation, vasomotions of bilateral retroauricular arteries which were caused by the stimulation of left great auricular nerve were observed. As shown in Fig. 7 a and 7 b, left retroauricular artery always dilated but right retroauricular artery either stayed unchanged (Fig. 7 a) or constricted (Fig. 7 b).

It is inferred that this constriction might be produced by some humoral vasoconstrictor substance, and the full explanation of this will be given in the author's separate report.

5) *The effect of eserine on the vasomotion induced by electrical stimulation*

The purpose of this experiment was to investigate on the nature of the active vasodilator nerve whose existence was suggested in the experiment 4. Eserine was used in this experiment because atropine at least on rabbits was undesirable to be used.[24] This experment was performed in the normal rabbit and the rabbit sympathectomized in the right ear.

i) Normal rabbit:

When left great auricular nerve was stimulated, the dilatation of bilateral retroauricular arteries was observed but in the contralateral artery was less as shown in Fig. 8. And then 0.2 mg/kg of body weight of eserine was injected intravenously at a very slow rate. About 15 min. after injection when the rabbit became still and the slight irregular vasomotion caused by eserine disappeared, left great auricular nerve was stimulated again with the same strength. The result is shown in Fig. 8.

Vasodilatation was seen bilaterally both before and after the injevtion, but its degree was greater bilaterally after the eserine injection. The vasodilatation of the right retroauricular artery was smaller than that of the left both before and after the injection. This phenomenon may be explained that

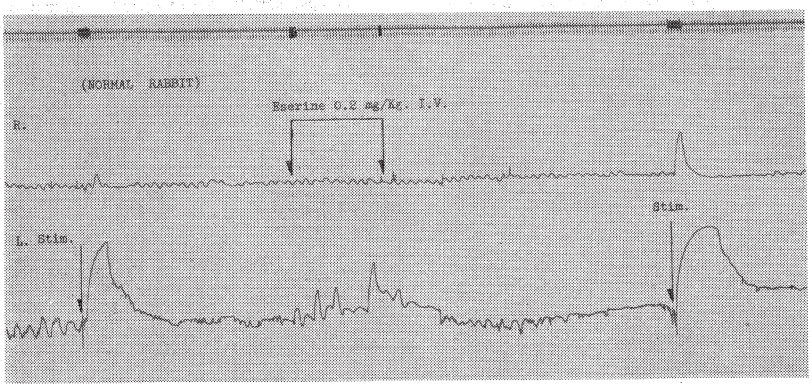


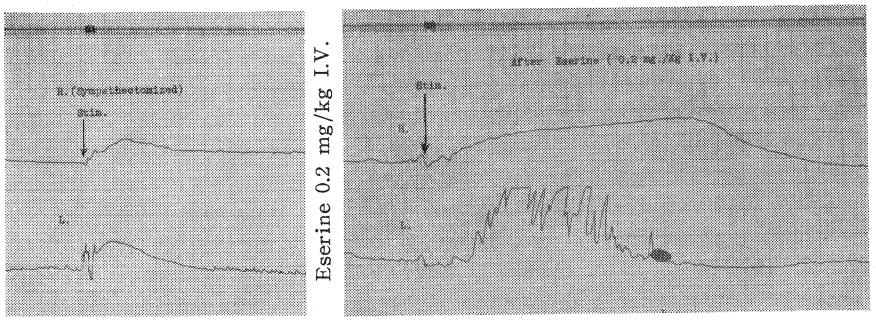
FIG. 8. The comparison of the vasomotion of bilateral retroauricular arteries with the stimulation of the left great auricular nerve before and after eserine administration.

stimulation impulse is less easily conveyed to the opposite side to the stimulated side[13] just as the results in experiments 1 and 3, comparing occnrrences of vasodilatation.

ii) The rabbit sympathectomized on the right ear:

Similar experiments were done in the rabbits sympathectomized in the right ear. The right great auricular nerve was stimulated. After bilateral vasodilatation was observed, eserine (0.2 mg/kg) was injected intravenously in the same way in the preceding experiment (as shown in Fig. 9 a and 9 b). Thereafter the nerve was stimulated again with the same strength. The results are shown in Fig. 9 a and 9 b.

An increase in degree of vasodilatation was seen after eserine injection in the majority of the cases (Fig. 9 a), but in some of the cases, the increase was seen only in the stimulated side without any noticeable alteration in the opposite side (Fig. 9 b). There were a few cases in which the vasodilatation even before eserine was not apparent. In this case, the effect of eserine was not observed. Except for those cases, the increase in degree of vasodilatation was clearly seen after eserine.



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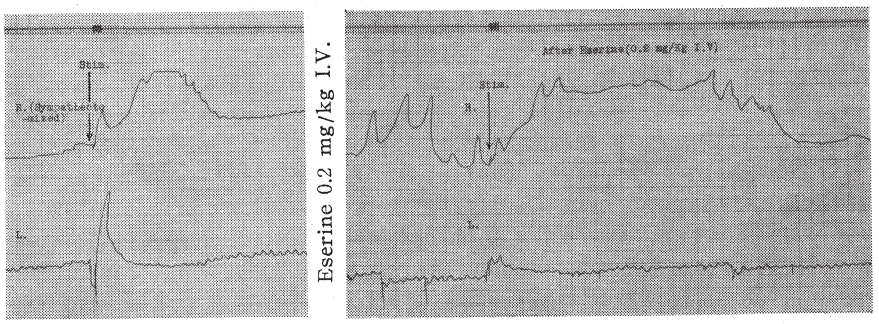

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FIG. 9. The comparison of the vasomotion of bilateral retroauricular arteries with the stimulation of the right great auricular nerve (sympathectomized) before and after eserine administration.

DISCUSSION

1. The method of sympathectomy in rabbit's ear

Sympathetic nerve fibres in the rabbit's ear are almost supplied from superior cervical ganglia but some of them are supplied from stellate ganglia directly. Umemoto[18] removed only superior cervical ganglia for his experiment on the vasomotor center. But for the purpose of complete sympathectomy from rabbit's ear, the stellate ganglia must be removed as well. Therefore, in these experiments right superior cervical and stellate ganglia were removed at least 7 days before the experiment. Usually the peripheral sympathetic nerve fibres are completely degenerated within a week after the operation.

2. RPPG used for recording of vasomotion

In his experiment on the vasomoter center, Umemoto[18] used numbers of drops of perfused solution per minutes as index of vasomotion. In the present experiment RPPG[20] was used for recording of vasomotion, which made continuous, sensitive, and direct recording possible. This method is usually not adequate for recording the vasomotion of a single artery, because it records the vasomotion of the accompanying vein as well.[1] However, the retroauricular artery runs alone in the midline towards the tip of the ear in rabbits, and therefore it is possible to record only the vasomotion of the artery.

3. The role of sympathetic on vasomotion

Folkow *et al.*[1] observed that in cross-circulation experiment on a cat and a dog, vasomotion of hind limbs disappeared after sympathectomy on abdominal trunk, and explained that a constant tone was maintained in the peripheral vessels and vasoconstriction occurred when the tone was raised with some stimulation, and vasodilatation occurred when the tone was lowered. But in the present experiments 1 and 3, clear vasomotion (vessel reaction) with electrical stimulation was observed even after sympathectomy. It can not, however, be denied that sympathetic has a great role in vasodilatation, when the occurrences of vasodilatation before and after sympathectomy is compared.

4. Active vasodilator nerve via posterior root

Bayliss[4,5] observed that the stimulation of the peripheral end of a cut sensory nerve resulted in vasodilatation of the area of the skin supplied by the nerve, and he inferred that this phenomenon was caused by antidromic impulse conduction of a sensory nerve. Since then Langley[6,7] and many other investgiators[8,9,10,11,12,13] have observed the same antidromic impulse conduction, and Holton and Perry[14,15,16] observed the vasodilatation caused with electrical stimulation of sympathectomized great auricular nerve, and they also attributed this phenomenon to the antidromic impulse conduction of a sensory nerve. However, Kuré *et al.*[17] ascertained that there were not only sensory nerve fibres but also parasympathetic fibres in the dorsal roots. Therefore, there are two possible explanations for the vasodilatation after stimulation of the peripheral end of a severed nerve, namely 1) the antidromic impulse conduction via sensory nerve fibres, and 2) the orthodromic impulse

conduction via parasympathetic fibres. It would be more natural from the neurophysiological point of view to suppose orthodromic impulse conduction as the explanation. In the experiment 1, the vasodilatation was observed when the sympathectomized nerve was stimulated, and the existence of active vasodilator nerve was suggested. Further, it was observed on the record of vasomotion of bilateral retroauricular arteries that stimulation impulse was conveyed to the opposite side of the stimulation. In the experiment 3, electrical stimulation of an intact (left) auricular nerve caused dilatation of the sympathectomized, contralateral (right) retroauricular artery. From this phenomenon, it would be possible to suppose that active vaosilatation exist besides the passive one due to lowering of sympathetic tone. And thus, the experiment 4 was performed in order to decide whether the nerve fibre is contained in the ventral root or in the dorsal root. In this experiment, the dorsal roots of the right C_2 and C_3 were cut on the rabbits in which vasodilatation was clearly observed after electrical stimulation of the left great auricular nerve. When a similar stimulation was given to the same nerve (left great auricular nerve) after severing of dorsal roots, an almost similar vasodilatation was observed in the left ear, but no dilatation occured in the right. This phenomenon may be considered as an evidence of existence of active vasodilator nerve fibres in those severed dorsal roots. And it may be concluded that vasodilatation is caused by the excitation of active vasodilator nerve via posterior roots (active vasodilatation), besides passive vasodilatation caused by the lowering of sympathetic tone.

5. Research on the nature of the active vasodilator nerve by using eserine

When a function of an organ is blocked by atropine, it is generally considered that the function is controlled with cholinergic fibre. But Dale[23] pointed out that the sensitivity to atropine is not indispensable condition of cholinergic nerve; for instance the parasympathetic vasodilatation in the salivary gland is relatively insensitive to atropine but is undoubtedly cholinergic. And Ellis[24] reported that rabbit's blood contains an esterase which destroys atropine and Holton and Perry[14] reported that the dosage of atropine is an important factor in the use of it on rabbits; a small dosis (1–100 μg intraarterial injection) antagonizes acetylcholine only temporarily and a large dosis (1 mg/kg or more intravenous injection) can abolish the effect of injected acetylcholine. And they stated that since the vasodilatation caused by an electrical stimulation of sympathectomized great auricular nerve was not blocked by atropine, this vasodilatation was due to antidromic impulse conduction via sensory nerve. But in this investigation atropine was not used because it was undesirable in the use at least on rabbits, and the effect of eserine on vasodilatation has been observed. In the experiment 5 comparing vasodilatation caused by the stimulation before and after eserine injection, increases in the degree and duration of the vasodilatation were clearly seen as shown in Fig. 8 and 9. Therefore, it may be considered that the active vasodilator nerve in the posterior root discussed in 4 is cholinergic.

6. Parasympathetic vasodilator nerve via posterior root

In the experiment in bull-frogs, Kotsuka[25] reported the existence of vasodilator nerve via posterior root, and he considered the nerve as sympathetic (sympathetic vasodilator nerve via posterior root), and further he considered that the nerve is cholinergic because the vasodilatation on stimulation of the nerve is increased with eserine and is blocked with atropine. But in this investigation, even after complete sympathectomy on a rabbit's ear, the vasodilatation of retroauricular artery was observed with the stimulation of great auricular nerve. From this observation, it would be more natural to consider that the nerve is not sympathetic but parasympathetic, as Kuré *et al.*[17] reported on the existence of parasympathetic in dorsal root of cervical cord. Therefore, it may be reasonably concluded from the results of the present series of experiments that vasodilatation consists of two genesis; the one is the passive vasodilatation caused with the lowering of sympathetic tone and the other is the active vasodilatation caused by parasympathetic vasodilator nerve via posterior roots.

SUMMARY

The rabbits was denervated sympathetically by removal of the stellate ganglia in addition to the removal of superior cervical ganglia and the vasomotion of retroauricular artery was recorded selectively, continuously and sensitively by using RPPG. Results were ollows:

1. Even after complete sympathectomy on a rabbit's ear, the vasodilatation of retroauricular artery could be induced by stimulation of great auricular nerve.

2. It is more difficult to induce vasodilatation in the sympathectomized ear by the electrical stimulation than to induce in the intact side.

3. Vasodilatation is caused passively by the lowering of sympathetic tone besides the active vasodilatation.

4. Though bilateral vasodilatation of retroauricular arteries can be caused by an unilateral stimulation of great auricular nerve, the vasodilatation of the opposite side to the stimulation is less frequently induced. Namely, a stimulation impulse is difficult to be conveyed to the opposite ear.

5. The vasodilatation is difficult to be induced in the ear with a stimulation of sciatic nerve.

6. The vasodilatation of the retroauricular artery can be induced in the sympathectomized (right) ear by stimulating on intact great auricular nerve in the opposite side (left).

7. After severing of dorsal roots of right-C_2 and C_3, the vasodilatation of right retroauricular artery was not observed on the stimulation of left great auricular nerve. In this case, vasoconstriction is seen occasionally, which is thought to be caused by some humoral substance.

8. The degree of vasodilatation with stimulation is clearly increased after eserine administration to a rabbit.

9. Active vasodilator nerve passes through the dorsal root and the nerve is cholinergic.

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REFERENCES

1. FOLKOW, B., G. STRÖM AND B. UVNÄS. *Acta Physiol. Scandinav.* **21**: 145, 1950.
2. FOLKOW, B. *Physiol. Rev.* **35**: 629, 1955.
3. STRICKER, S. S. S. B. *Akad. Wissen.* **74**: 173, 1870, (cited from FRUMIN, M. J., S. H. NGAI AND S. C. WANG. *Am. J. Phisiol.* **173**: 428, 1953).
4. BAYLISS, W. M. *J. Phisiol.* **26**: 173, 1901.
5. BAYLISS, W. M. *J. Phisiol.* **28**: 276, 1902.
6. LANGLEY, J. N. *J. Phisiol.* **57**: 428, 1923.
7. LANGLEY, J. N. *J. Physiol.* **58**: 49, 1923.
8. BARRON, D. H. AND B. H. C. MATHEWS. *J. Physiol.* **85**: 104, 1935.
9. TOENNIES, J. T. *J. Neurophysiol.* **1**: 378, 1938.
10. DUN, F. T. *J. Physiol.* **95**: 41, 1939.
11. SKOGLUND, C. R. AND B. UVNÄS. *Acta Physiol. Scandiuav.* **6**: 149, 1943.
12. GÖPFERT, H. F. *J. Physiol.* **133**: 433, 1956.
13. TREGEAR, R. T. *J. Physiol.* **142**: 343, 1958.
14. HOLTON, P. AND W. L. M. PERRY. *J. Physiol.* **114**: 240, 1951.
15. HOLTON, P. *J. Physiol.* **131**: 176, 1956.
16. HOLTON, P. *J. Physiol.* **145**: 494, 1959.
17. KURÉ, K. *ET AL. Quart. J. exp. Physiol.* **21**: 119, 1931.
18. UMEMOTO, K. *Folia Phvrmacol. Japon.* **55**: 739, 1959 (Japanese).
19. KANDA, Z. *ET AL. Folia Pharmacol. Japon.* **52**: 577, 1956 (Japanese).
20. SAWADA, M. *Respiration and Circulation* **6**: 417, 1958 (Japanese).
21. HERTZMAN. *Am. J. Physiol.* **124**: 328, 1938.
22. TAKAGI, K. AND T. NAGASAKA. *The Journal of Japanese College of Angiology* **2**: 93, 1962 (Japanese).
23. DALE. H. H. *Lancet* **1**: 1290, 1929.
24. ELLIS, S. *J. Pharmacol.* **91**: 370, 1947.
25. KOTSUKA, K. AND H. NAITO. *Acta Neurovegetativa* **4**: 454, 1962.