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Pharmacological Study on the Aortic Arch Baroreceptors in the Rabbit[1)]

Jun-ichi Ishiko and Hideomi Fukuda

Faculty of Pharmaceutical Sciences, Nagoya City University[2)]

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The afferent impulses from the aortic arch baroreceptor in the left aortic nerve of rabbits were recorded *in situ* and in the perfused preparation. Epinephrine, acetylcholine, lyoniol-B and ouabain excited the baroreceptor in both preparations. Propranolol and N-propyl ajmaline decreased the rate of afferent impulses in the *in situ* preparation, but had no influence in the perfused preparation. Possible mechanisms of these drugs affecting the impulse activity in the aortic nerve are discussed.

Baroreceptors in the carotid sinus and the aortic arch are the major sense organs which reflexly control the arterial blood pressure and the heart rate.[3–6)] Fewer studies about physiological and pharmacological properties of aortic arch baroreceptors have been performed than about carotid sinus receptors, chiefly because the technical difficulties involved in the isolation of the aortic arch precluded detailed analysis.

Bradycardia caused by ouabain is abolished by denervation around the aortic arch in the cat and rabbit.[7)] It, therefore, seems likely that the blood pressure response and the cardiac arrhythmias caused by ouabain are concerned with the reflex mechanism of baroreceptors in the aortic arch.

Recently, it was found by the present authors that lyoniol-B, one of the diterpenoids isolated from *Lyonia ovaliforia*, exerted a marked excitatory effect on the afferent activities of muscle spindle[8,9)] and the vagus nerve.[10)]

In most experiments, conclusions on baroreceptor reflexes were derived from measurements of some circulatory phenomena such as heart rate and blood pressure, which were the resultants of many complex interactions and therefore could not accurately reflect the neural function of baroreceptors. For this reason, it is significant to study more directly the contribution of afferent neural systems to the circulatory system.

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2) Location: *Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan.*
3) D.W. Bronk, *Proc. Soc. Exptl. Biol. Med.*, **28**, 1014 (1930).
4) W.W. Douglas and W. Schaumann, *J. Physiol.*, **132**, 173 (1956).
5) S. Homma and S. Suzuki, *Japan. J. Physiol.*, **16**, 31 (1966).
6) M.B. Kardon, D.F. Peterson, and V.S. Bishop, *Am. J. Physiol.*, **225**, 7 (1973).
7) H.F. Zipf and H. Ehrlicher, *Arch. Exptl. Pathol. Pharmakol.*, **212**, 529 (1951).
8) H. Fukuda, Y. Kudo, H. Ono, M. Yasue, J. Sakakibara, and T. Kato, *Chem. Pharm. Bull.* (Tokyo), **22**, 884 (1974).
9) H. Fukuda, Y. Kudo, and H. Ono, *Europ. J. Pharmacol.*, **26**, 136 (1974).
10) H. Ono, Y. Kudo, and H. Fukuda, *Japan. J. Pharmacol. Suppl.*, **22**, 101 (1972).

The present study was undertaken to investigate the effect of ouabain, lyoniol-B and some antiarrhythmic agents on afferent discharges from the aortic arch baroreceptors in the whole animal and perfused preparation in the rabbit. The rabbit may be a favoured animal for these experiments, since aortic nerves contain baroreceptor fibres but relatively few chemoreceptor fibres.[11,12]

Methods and Materials

Forty-two male rabbits weighing from 2.5 to 3.3 kg were used in this study.

Preparation A: Aortic Arch Baroreceptor *in Situ*——The animals were anesthetized with urethane (1.5 g/kg, *s.c.*), tracheotomized and ventilated with air. The aortic nerve was isolated about 20 mm in length from the vagosympathetic trunk. Exposed nerves were placed on platinum electrodes and covered with liquid paraffin. The distance between the electrodes was about 2 mm. The afferent impulses were displayed on a cathode-ray oscilloscope (Nihonkohden VC-7) through a biophysical preamplifier (Nihonkohden AVB-2). The same impulses were then transformed into square waves and fed into an integrator, the output of which was recorded on an ink-writing oscillograph; electrocardiograms and femoral blood pressure were simultaneously measured. Drugs were injected into the left femoral vein through a cannula.

In a few experiments, changes in afferent discharges from the aortic arch baroreceptors at various levels of arterial blood pressure were investigated. In this case, arterial blood pressure was changed by the stepwise removal and retransfusion of blood through a reservoir connected to the abdominal aorta.

Preparation B: Perfused Aortic Arch Preparation——The arrangement was approximately the same as that described by Angell James.[13] The diagram is shown in Fig. 1. The animals were anesthetized with urethane (1.5—2.0 g/kg, *s.c.*). A tracheotomy was performed and positive pressure ventilation was maintained with a respiration pump at a frequency of 30 strokes/min. A midline thoracotomy was performed. The right and left subclavian arteries, the internal mammary arteries and bilateral carotid arteries were prepared for subsequent ligation. The descending thoracic aorta was cannulated with a polyethylene cannula, and then the right and left subclavian arteries and internal mammary arteries were ligated. The preparation was perfused through a cannula which was passed through the wall of the left ventricle so that its tip lay in the ascending aorta. The bilateral carotid arteries were ligated. The effluent passed out *via* the descending aorta cannula, and the flow was controlled by a screw-clamp type resistance. The perfusion fluid was a modified Krebs-Henseleit solution (NaCl 117.6 mM, KCl 5.63 mM, $CaCl_2$ 1.27 mM, $MgSO_4$ 1.20 mM, NaH_2PO_4 1.17 mM, $NaHCO_3$ 25.0 mM and glucose 5.55 mM). The fluid was equilibrated with 95% oxygen and 5% carbon dioxide, giving a pH of 7.4. The flow through the preparation was maintained at about 5 ml/min. The temperature of perfusion fluid was maintained at 38—39° in a thermostatically controlled water bath.

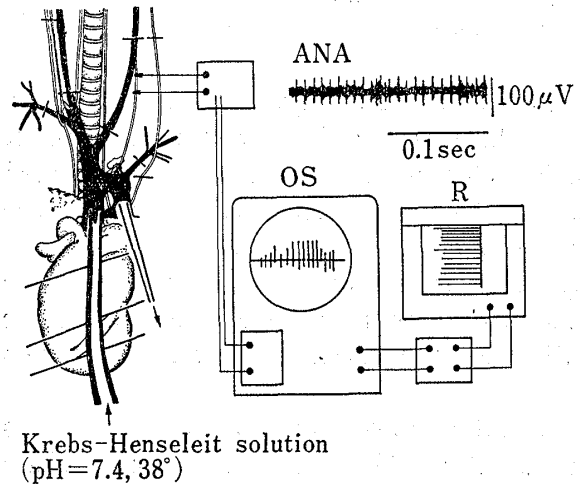

Fig. 1. Diagram showing the Isolated Perfused Aortic Arch and Aortic Nerve Trunk of the Rabbit and indicating the Site of the Ligature and Cannulae

ANA: aortic nervous activity,
OS: oscilloscope
R: DC-recorder

Nerve potential recordings were made in according to the method described in preparation A. Intra-aortic perfusion pressure was simultaneously measured. Drugs were injected into the ascending aorta through a cannula.

Drugs used were acetylcholine chloride (Ovisot, Daiichi), acetic acid (Wako Pure Chemical), epinephrine–HCl (Adrenalin, Sankyo), ouabain (g-strophantin, Merk), N-propyl ajmaline dihydrogentartrate (Nihon Chemiphar) and lyoniol-B (deacetyl lyoniol-A, supplied by Prof. M. Yasue, Faculty of Pharmaceutical Sciences, Nagoya City University).

11) W.W. Douglas, J.M. Ritchie, and W. Schaumann, *J. Physiol.*, **132**, 187 (1956).
12) J.P. Chalmers, P.I. Korner, and S.W. White, *J. Physiol.*, **188**, 435 (1967).
13) J.E. Angell James, *J. Physiol.*, **214**, 65 (1971).

Results

In preparation (A), two different types of afferent impulses appeared synchronously with the heart beat; high amplitude impulses which occurred following the R spike of the ECG and low amplitude impulses which occurred following high amplitude impulses (Fig. 2-(1)). The onset of aortic nervous activity preceded that of carotid nervous activity by 25 msec (Fig. 2-(2)). Removal of 20 ml of blood in one of the femoral artery caused a decrease in blood pressure by 30 to 40 mmHg; at this time, there was a marked decrease in the frequency of the high amplitude impulses. This result suggests that the changes in afferent discharges depend on those in blood pressure.

In preparation (B), acetic acid (0.5N), known to destroy the chemoreceptors,[14] did not influence impulse activity in baroreceptor fibres from the aortic arch. This result confirmed the finding of Chalmers, *et al.*[12] that relatively few chemoreceptor fibres originate from the aortic region in the rabbit.

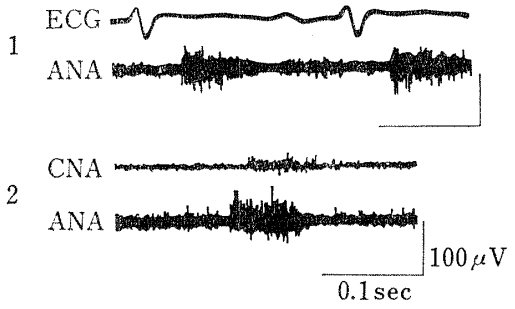

Fig. 2. Recordings of the Left Aortic Nervous Activity, Carotid Sinus Nervous Activity and ECG

(1) Upper and lower tracings are ECG (II) and the original record obtained from the left aortic nerve trunk, respectively.
(2) Upper and lower tracings are records obtained from the carotid sinus nerve trunk and the left aortic nerve trunk, respectively.
CNA: carotid sinus nervous activity,
ANA: aortic nervous activity

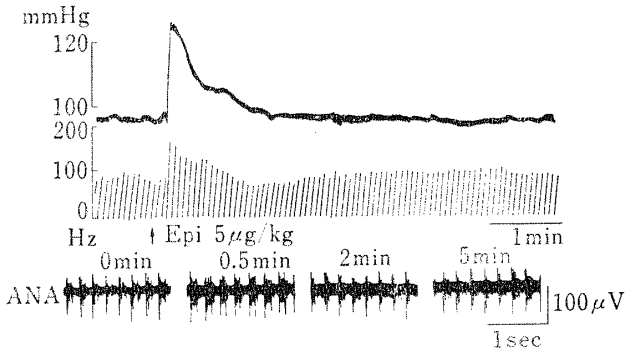

Fig. 3. Effect of Epinephrine on the Impulse Discharge from the Left Aortic Nerve and Blood Pressure

upper tracing: blood pressure, middle tracing: integrated aortic nervous activity, lower tracing: original record of aortic nervous activity, and ANA: aortic nervous activity

I) Epinephrine-HCl

In preparation (A), epinephrine-HCl (5 μg/kg, *i.v.*) caused an increase in blood pressure by 25—30 mmHg and an increase in the frequency of aortic nervous impulses by 80—150%. There was a marked increase in the frequency of high amplitude impulses synchronous with cardiac systole (Fig. 3). In preparation (B), perfusion of epinephrine-HCl (2 μg) caused an increase in the rate of discharge.

II) Acetylcholine Chloride

In preparation (A), acetylcholine chloride (10 μg/kg, *i.v.*) lowered blood pressure by 25 mmHg and reduced the frequency of discharges by 50%. When impulses synchronous with cardiac rhythm were abolished finally, another kind of impulse was found to appear continuously (Fig. 4). In preparation (B), acetylcholine chloride (1—10 μg) caused no influence on the afferent activity in three experiments and a slight increase in two experiments.

III) Ouabain

In preparation (A), ouabain (5—10 μg/kg, *i.v.*) caused an increase in the frequency of the afferent discharge; a maximum increase in the rate of discharge was about 200%. The dose

14) J.A. Quest and R.A. Gillis, *J. Pharmacol. Exptl. Therap.*, **177**, 650 (1971).

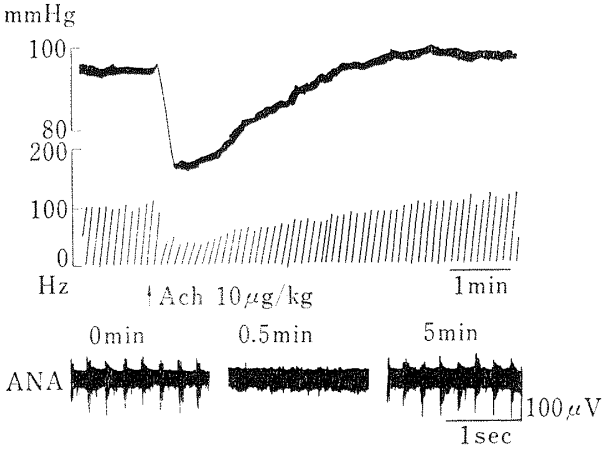

Fig. 4. Effect of Acetylcholine on the Impulse Discharge from the Left Aortic Nerve and Blood Pressure

upper tracing: blood pressure, middle tracing: integrated aortic nervous activity, lower tracing: original record of aortic nervous activity, and ANA: aortic nervous activity

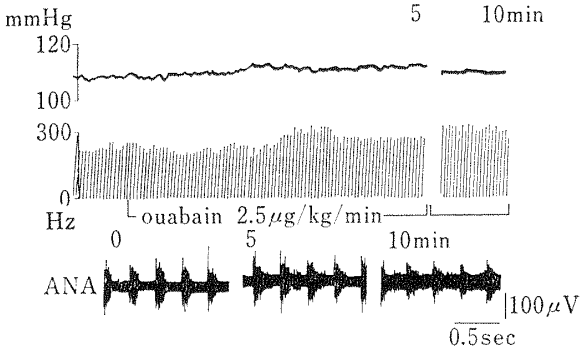

Fig. 5. Effect of Ouabain on the Impulse Discharge from the Left Aortic Nerve and Blood Pressure

upper tracing: blood pressure, middle tracing: integrated aortic nervous activity, lower tracing: original record of aortic nervous activity, and ANA: aortic nervous activity

also caused an increase or a slight decrease in blood pressure. The increased aortic nervous activity still remained even when the blood pressure returned to the control level. To exclude interference with blood pressure changes induced by the drug, a further experiment was carried out in which ouabain was infused intravenously at a rate of 2.5 μg/kg/min by means of an infusion pump. Ouabain caused none or only a slight increase (5 mmHg) in blood pressure, although the frequency of afferent discharges caused a maximum increase of 50—100 % in all of 4 experiments. An increase in the frequency of afferent discharges was observed 2—3 min after starting the ouabain infusion; 10—20 min after, small amplitude impulses appeared continuously independent of the heart beat (Fig. 5).

Also in preparation (B), 10 μg of ouabain caused a marked increase in the rate of discharges, lasting for 5 min. Thus, the increase in the rate of afferent discharges caused by ouabain may be produced at least in part by the direct action of the drug on the baroreceptor (Fig. 6-(1)).

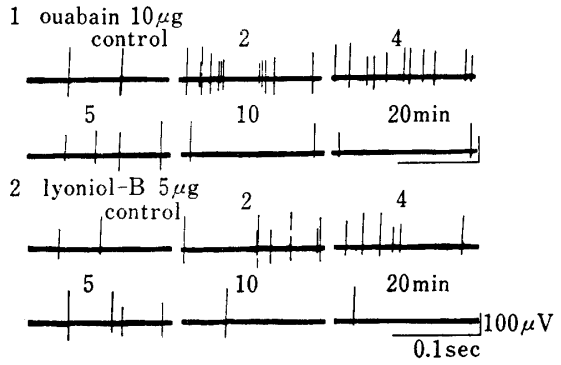

Fig. 6. Effect of Ouabain and Lyoniol-B on the Impulse Discharge from the Left Aortic Nerve in the Perfused Aortic Arch Preparation

1) changes in aortic nervous activity before and after administration of ouabain (10 μg),
2) changes in aortic nervous activity before and after administration of lyoniol-B (5 μg). Intra-aortic perfusion pressure was maintained constant at 80 mmHg.

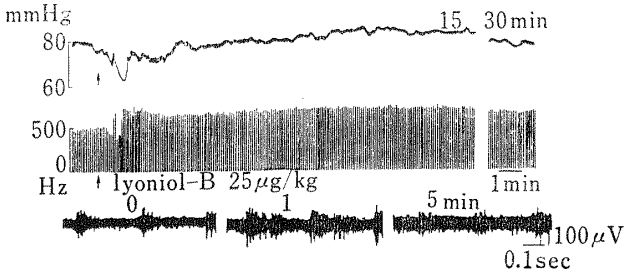

Fig. 7. Effect of Lyoniol-B on the Impulse Discharge from the Left Aortic Nerve and Blood Pressure

upper tracing: blood pressure, middle tracing: integrated aortic nervous activity, lower tracing: original record of aortic nervous activity, and ANA: aortic nervous activity

IV) Lyoniol-B

In preparation (A), lyoniol-B (25 μg/kg, *i.v.*) caused a persistent increase in the frequency of impulses in the aortic nerve, while causing a transient decrease in blood pressure. The firing time of high amplitude impulses prolonged within 1—2 min and afferent discharges synchronous with the cardiac rhythm became continuous (Fig. 7).

In preparation (B), 5 μg of lyoniol-B caused a marked increase in the rate of discharge, lasting for 5—10 min (Fig. 6-(2)).

V) Propranolol and N-Propyl Ajmaline

In preparation (A), propranolol (1 mg/kg, *i.v.*) decreased slightly the frequency of impulses accompanied with a transient decrease in blood pressure. A prolongation of the firing time of high amplitude impulses and an increase in the amplitude of low amplitude impulses with a decrease in the heart rate were observed.

N-propyl ajmaline (0.5 mg/kg, *i.v.*) decreased the frequency of impulses (60—80%) accompanied by a marked decrease in blood pressure, and 15—20 min after, the impulse activity returned to the control level.

In preparation (B), propranolol and N-propyl ajmaline at doses of 10—100 μg caused no influences on the impulse activity.

Discussion

The present study has shown several characteristics of afferent discharges from the aortic arch baroreceptors of rabbits: a) the discharges are driven synchronously with heart beat; the high frequency and high amplitude discharges which occur during ventricular systole and the succeeding low amplitude discharges are observed; the onset of aortic nervous activity precedes that of carotid nervous activity by 25 msec; b) with decreasing blood pressure by bleeding, the frequency of discharges decreases; and c) the discharges are unaffected by the perfusion of acetic acid, which is known to destroy the chemoreceptor, supporting Chalmers's finding[12] that relatively few chemoreceptor fibres originate from the aortic region in the rabbit.

The discharges were markedly influenced by various drugs. Possible mechanisms of drug action affecting the impulse activity in the aortic nerve should include the following: a) response of the baroreceptors to change in blood pressure, b) alteration in the tension of the arterial wall, in which baroreceptors are located, and c) alteration in the excitability of the impulse-generating mechanism in the baroreceptor.

Epinephrine caused an increase in the afferent discharges associated with an increase in blood pressure. As it was observed that epinephrine significantly increased discharges with constant pressure in the perfused aortic arch preparation, other causes than change in blood pressure must be considered. It was reported that epinephrine produced a contraction of the smooth muscle of the aortic wall and reduced the aortic diameter (Aars).[15] One explanation of the increase in the discharges caused by epinephrine would be that the increased excitability of the impulse-generating mechanism in the baroreceptor exceeded the decrease in discharges by narrowing of the aortic diameter.

An alternative explanation may be more probable: epinephrine contracts a certain site of the arterial wall, and would be expected therefore to extend the receptor area and thereby increase the baroreceptor activity. This appears possible if one refers to the gamma-bias controlling the intrafusal fibres in the muscle spindles of the skeletal muscle. The finding (Sampson and Mills)[16] that the sympathetic nervous system controls the sensitivity of the baroreceptors may suggest a similarity to gamma-bias.

15) H. Aars, *Circ. Res.*, **28**, 254 (1971).
16) S.R. Sampson and E. Mills, *Am. J. Physiol.*, **218**, 1650 (1970).

Ouabain caused an increase both in the experiment in which the blood pressure was kept constant by the slow injection of the drug and in the perfusion experiment. Broekaert and Godfraind[17] have shown that ouabain induces a contraction of isolated arteries in rabbits. It may, therefore, be thought that the increased discharge by ouabain is produced at least partly by the causes, such as direct actions of ouabain on the arterial diameter and the baroreceptor sensitivity.

Very recently, Quest and Gillis[18] observed that digitalis increased the spontaneous firing of the carotid sinus nerve in the perfused carotid sinus preparation of cat, and concluded that digitalis could directly alter the sensitivity of baroreceptor and produce significant changes in carotid sinus nerve activity.

Acetylcholine abolished the discharges synchronous with a cardiac rhythm during the period of decreased blood pressure, but another kind of impulse was found to appear continuously. Sato and Tasaki[19] have already reported a similar observation. Also in the perfused aortic arch preparation, acetylcholine excited the baroreceptor. In this connection, it has already been suggested by Diamond[20] that the sensory terminal in the carotid sinus is specific with regard to acetylcholine excitation. Thus, the cholinergic mechanism may be thought to contribute to the initiation of afferent discharges from aortic arch baroreceptors as well as from the carotid sinus.

Lyoniols exerted a marked excitatory effect on the muscle spindle and on vagal afferents.[8–10] In the present study, lyoniol-B persistently increased afferent discharges without an increase in blood pressure. The present authors feel that lyoniol-B has a direct action on the impulse-generating mechanism in the baroreceptor in the same way as was found in the muscle spindle (Fukuda, *et al.*).[8,9]

Propranolol and N-propyl ajmaline caused a decrease in the frequency of discharges accompanied with a decrease in blood pressure in the *in situ* experiment; but, in the perfused preparation, a dose of 100 μg exerted no influence. Thus, it seems likely that changes in afferent discharges by both drugs are mainly associated with a decrease in blood pressure.

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17) A. Broekaert and T. Godfraind, *Arch. Intern. Pharmacodyn.*, 203, 393 (1973).
18) J.A. Quest and R.A. Gillis, *Circ. Res.*, **28**, 254 (1974).
19) M. Sato and I. Tasaki, *Japan. J. Physiol.*, **1**, 173 (1950).
20) J. Diamond, *J. Physiol.*, 130, 513 (1955).