## Notes

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# A Simple Method for the Measurement of the Cardiac Afferent Activities as affected by Drugs, using the Perfused Heart of the Bullfrog[1)]

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Afferent activity from baroreceptors in the isolated heart of the bullfrog was recorded. Two types of volley were observed during the period of the cardiac cycle: a lasting volley of discharges accompanying the QRS complexes in electrocardiogram and a short volley of discharges following immediately after the P wave. Epinephrine, veratridine and ouabain caused an increase in the rate of afferent discharges from baroreceptors in the heart, whereas acetylcholine and ajmaline caused a reduction in the rate. Thus this method allowed a simple measurement of the effect of drugs on the cardiac afferent activities.

Afferent discharges from the cardiovascular region are responsible for the cardiac depressor reflex and cardiac pain in mammals.[3,4)] Recently, it has been proposed that syncope in aortic stenosis in man results from a large increase of the contribution of left ventricular baroreceptors to vagal tone which results in reflex bradycardia and hypotension.[5)]

In amphibians, afferent unitary discharges from the mechanoreceptors in the ventricle, the atrium, and the sinus have been found.[6)] It was, therefore, of interest to examine the relationship between afferent discharges and cardiac cycle in the perfused heart of the bullfrog. Modification of afferent discharges by ouabain and some other drugs acting on the circulatory system was also investigated.

### Methods and Materials

The bullfrog (Rana catesbiana) of either sex weighing 200—300 g was used. The heart was excised from the body together with the cardiac branches of the vagosympathetic nerves; and then the heart was perfused with amphibian Ringer's solution composed of NaCl 115 mM, KCl 2.7 mM, $CaCl_2$ 1.8 mM, $NaHCO_3$ 3.0 mM, and glucose 5.5 mM. The cardiac nerve branch was placed on a pair of platinum electrodes and immersed in liquid paraffin to prevent drying of the nerve fibres. The afferent impulses were amplified with a Nihinkohden AVB-2 amplifier and displayed on a Nihonkohden VC-7 oscilloscope. The same impulses were then transformed into square waves and fed into an integrator, the output of which was recorded by an ink-writing oscillograph (Fig. 1).

Drugs used were acetylcholine chloride (Ovisot, Daiichi), ajmaline (Gilurytmal, Nihon-Chemiphar), *dl*-epinephrine hydrochloride (Adrenalin, Sankyo), ouabain (Merck) and veratridine (Aldrich Chemicals). All were dissolved in amphibian Ringer's solution.

## Results

### 1) Relationship between Afferent Discharges and the Cardiac Cycle

Two types of volley of afferent discharges synchronous to the heart beat were observed in the perfused heart preparation (Fig. 1): a lasting volley of discharges with high frequency

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2) Location: *Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan.*
3) J.C.G. Colerridge, A. Hemingway, R.L. Holmes, and R.J. Linden, *J. Physiol.* (London), **136**, 174 (1957).
4) A.S. Paintal, *Ergebn, Physiol.*, **52**, 74 (1963).
5) A.M. Johnson, *Brit. Heart J.*, **33**, 1 (1971).
6) T. Kolatat, K. Kramer, and N. Mühl, *Pflügers Arch. ges. Physiol.*, **264**, 127 (1957).

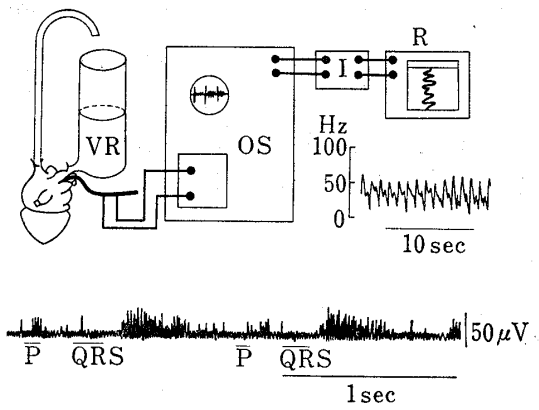


Fig. 1. Spontaneous Afferent Discharges from the Perfused Heart of the Bullfrog

Upper figure shows schematic illustration of the perfusion method. VR: venous reservoir, OS: oscilloscope, I: integrator, R: recorder
Lower figure shows afferent discharges synchronous with heart beats.

appeared during the latter period of ventricular ejection, the subsequent period of ventricular diastole, and the period of atrial filling, whereas a short volley of discharges with low frequency followed immediately after the P-wave which coincided with auricular systole.

### 2) Relationship between Afferent Discharges and Perfusion Pressure

With increasing the level of Ringer's solution in the venous reservoir from 0.5 $cmH_2O$ to 1.0, 2.0 and 3.0 $cmH_2O$, the maximum frequency of the afferent discharges was increased to approx. 153, 260 and 290%, respectively.

### 3) Effect of Drugs on Afferent Discharges

**a) Epinephrine**——Epinephrine-HCl (a final concentration of $1\times10^{-7}$ g/ml) produced an increase in the rate of afferent discharge, especially of a lasting volley, as well as an increase of the cardiac contractile force of the heart. The maximum frequency of the discharges was increased to 150%. However, the firing duration was decreased with an increase in the heart beat (Fig. 2a).

**b) Acetylcholine**——Acetylcholine chloride ($5\times10^{-7}$ g/ml) caused a cardiac arrest and then an abolition of afferent discharges 3 min after application. It was clear that the abolition of discharges was dependent upon the cardiac arrest (Fig. 2b).

**c) Veratridine**——Veratridine ($5\times10^{-6}$ g/ml), an established stimulant of the sensory endings,[7] produced spontaneous irregular discharges and significantly increased the rate of afferent discharge; although the amplitude of discharges and cardiac rhythms were little affected (Fig. 2c).

**d) Ouabain**——Ouabain ($1\times10^{-5}$ g/ml) produced the cardiac ventricular tachycardia and simultaneously increased the rate of discharge to 4—5 times the control level. After the

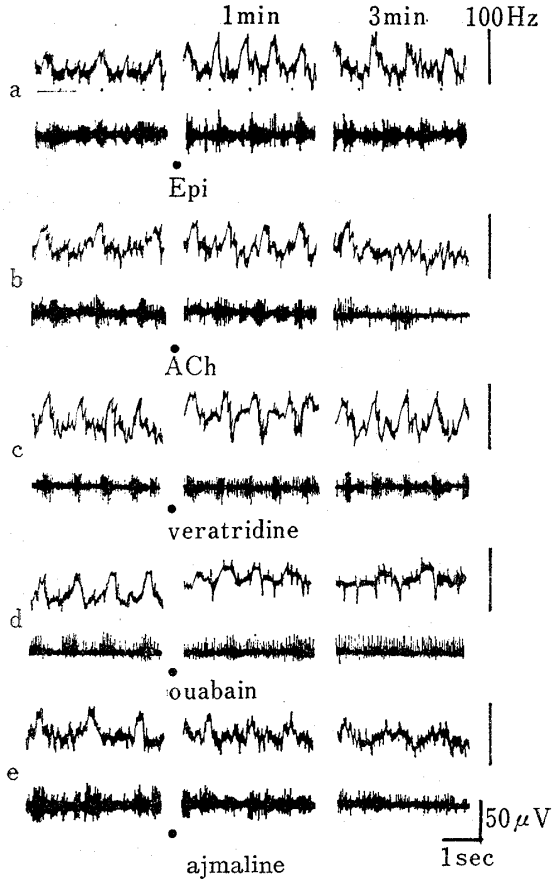


Fig. 2. Effect of Epinephrine, Acetylcholine, Veratridine, Ouabain and Ajmaline on Afferent Discharges from the Perfused Heart

upper trace: the impulse frequency of discharges recorded by using an integrator and an ink-writing oscillograph. lower trace: an original recording of the discharges. Dots indicate when drugs were administered into the perfusion cannula.
*a*) epinephrine-HCl $1\times10^{-7}$ g/ml,
*b*) acetylcholine chloride $5\times10^{-6}$ g/ml,
*c*) veratridine $5\times10^{-6}$ g/ml,
*d*) ouabain $1\times10^{-5}$ g/ml,
*e*) ajmaline $1\times10^{-6}$ g/ml

7) A. Jarisch and H. Richter, *Arch. Exptl. Pathol. Pharmakol.*, **193**, 355 (1939).

application of ouabain, the firing duration was prolonged; and then appearance of the afferent discharges became irregular. These discharges were independent of the cardiac rhythms; at this time, right atrial pressure recorded simultaneously remained unchanged. Larger doses of $1\times10^{-5}$ g/ml or more produced a temporary increase in the rate of discharge, and then abolished discharges while causing a cardiac arrest (Fig. 2d).

e) **Ajmaline**——Ajmaline ($1\times10^{-7}$ g/ml) gradually decreased the maximum frequency of discharge. The onset of the decrease in the discharges slightly preceded that of the decrease in cardiac contractions. About 3 min after the application of $10^{-6}$ g/ml cardiac arrhythmias appeared, followed by cardiac arrest; at the same time, an abolition of afferent discharges was observed (Fig. 2e).

## Discussion

In the present study using the perfused heart of the bullfrog, two types of afferent discharges were observed during the atrial contraction and the atrial filling, as was found by Kolatat, *et al.*[6] The frequency of afferent discharges was increased by the elevation of the level of Ringer's solution in the venous reservoir, indicating that afferent discharges may originate from the baroreceptors of the atrium and ventricle in the heart of the bullfrog.

The change in the afferent discharges by epinephrine and acetylcholine was associated with that in contraction of the cardiac muscle. It seems probable that the excitatory effect of epinephrine on atrial and ventricular receptors results from its positive inotropic and chronotropic actions. Similar result has been reported in the dog.[8] It has been demonstrated that acetylcholine excited directly the carotid chemoreceptors and baroreceptors.[9] In the heart, however, acetylcholine is well known to have negative inotropic and chronotropic actions. Therefore, it seems likely that changes in the afferent discharges by acetylcholine are associated mainly with the negative inotropic and chronotropic actions.

Veratridine produced an increase in the rate of the afferent discharge. Veratrum alkaloids, noted for producing the Bezold-Jarisch effect, are apparently the substances which can stimulate sensory endings of all medullated fibres. Paintal[10] reported that veratrum alkaloids greatly stimulated the receptors in the heart of cats.

Ouabain produced an increase in the rate of afferent discharge, and the discharges irregularly appeared, independently of the cardiac rhythm. Ajmaline decreased the rate of afferent discharge.

Cardiac afferent activities were found to be susceptible to drugs in this preparation. Afferent signals from the heart may play an important role in control of the cardiac rhythm. It seems reasonably clear that the excitation or depression of cardiac receptors caused by drugs influences the physiological conditions of the heart. In conclusion, the method reported here allowed a simple measurement of the effect of drugs on the cardiac afferent activities.

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10) A.S. Paintal, *J. Physiol.* (London), **135**, 486 (1957).