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**Short Communication**

# Reformative Cannula Electrodes for His Bundle Electrogram Recording in Isolated Rat Hearts

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## Abstract

**His bundle electrogram (HBE) recording is an important method for the study of the atrioventricular conduction system. However, the current HBE recording methods in isolated animal hearts have some disadvantages, such as unstable recording due to the difficulty in fixing electrodes as a result of intense heart beat, the small amplitude of the His signal or the possibility to destroy the integrity of heart structure. To overcome these disadvantages, we designed and manufactured reliable, inexpensive and easy-made bipolar cannula electrodes, which combine the functions of Langendorff-perfusion aortic cannula and recording electrodes. With the cannula electrodes, the operation of HBE recording becomes easier and clearer; hence, more stable recordings can be obtained in isolated rat hearts.**

**Key Words: cannula electrodes, his bundle electrogram, langendorff-perfusion heart, rat**

## Introduction

His bundle electrogram (HBE) recording is an important method for the study of atrioventricular conduction system (AVCS). The HBE recording in isolated animal heart usually involves three methods in Langendorff-perfusion heart model: [a] Recording electrodes were positioned in the AV valve plane of epicardiac surface (4); [b] The endocardial surface of the right atrium was exposed *via* an incision along the anterolateral atrioventricular groove. The recording unipolar electrode was placed on the endocardium near the apex of the triangle of Koch (1). [c] A unipolar polytetrafluorethylene-coated stainless steel electrode was placed in the AV nodal area through a small right ventricular incision adjacent to the right atrium (3). However, these methods can not give a stable recording due to the difficulty in fixing electrode as a result of intense heart beating. In addition, the amplitude of the His signal is small in the first method and the integrity of heart structure could be destroyed in the later two methods.

To overcome these disadvantages, we designed and manufactured reliable, inexpensive and easy-made bipolar cannula electrodes referred to the method of Zhang *et al.* (5), which combined the functions of Langendorff-perfusion aortic cannula and recording electrodes. With the cannula electrodes, the operation of HBE recording becomes easier; hence, and clearer and more stable recordings can be obtained in isolated rat hearts.

## Materials and Methods

*Manufacture of Cannula Electrode (Fig. 1)*

A rigid plastic tube with a length of 3.0 cm, an out-diameter of 2.0 mm and an inner-diameter of 1.8 mm, was chosen for aorta cannula. One end of the tube was sheared to a wedge-shape, and two pinholes with separation of 1.0 mm were drilled with a needle at the site that is just 2 mm to the tip of the wedge.

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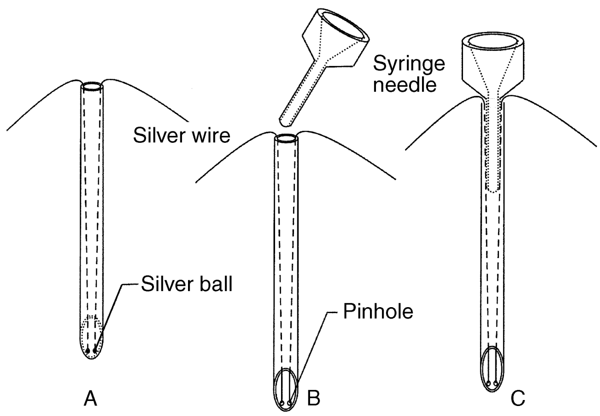


Fig. 1. Schematic diagram of the cannula electrodes manufactured.

Two identical silver wires with a length of 15.0 cm and a diameter of 0.2 mm were coated with insulated polyimide enamel (SSIM406, Shanghai Shengran Insulation Materials Co. Ltd, Shanghai, China), and one end of each wire was burnt to a silver ball. Then each wire was threaded into one pinhole from the outer-wall of the tube, making the two balls block the pinholes. The other two ends of the wires went through the tube and connected to the input preamplifier for recording. The silver balls are used as probes for His bundle signals. A syringe needle, with an outer-diameter of 1.3 mm and a length of 1.0 cm and coated with super glue (Shuizhong Jiao, Guangzhou Dongfeng Chemicals Industrial Co. Ltd., Guangdong, China), was partially plugged into the tube from its flat end so as to seal it. The sticking-out end of the syringe needle is fixed to the Langendorff apparatus.

*Recording Parameters*

Electrical signals were continuously monitored and recorded on a hard disc in a personal computer after digitization through a multichannel physiological signal recording system (RM6240BD, Chengdu Instrument Factory, Sichuan, China) at a sampling frequency of 1 kHz. This recording system contains a preamplifier with 100 MΩ input impedance. The HBE signals were amplified 5,000 times and the epicardium ECG were amplified 500 times with a time constant of 0.2 s (high pass filtering 0.8 Hz) and low pass filtering 100 Hz.

*Langendorff-Perfused Heart Model and Electrophysiologic Recording*

Male Sprague-Dawley rats (200-300 g body weight) were anaesthetized with sodium pento-barbitone (50 mg/kg, i.p.) and heparinized (250 units, i.p.). The rat heart was then excised carefully and mounted immediately on the home-made cannula electrodes fixed to Langendorff apparatus and perfused retrogradely *via* the aorta with Tyrode solution containing (in mM): NaCl 137.0, KCl 5.4, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, $NaH_2PO_4$ 0.33, $CaCl_2$ 1.8 and glucose 10. The solution was continuously bubbled with 95% $O_2$ and 5% $CO_2$ to give rise to a pH of 7.4 and was maintained at 37°C with a water bath.

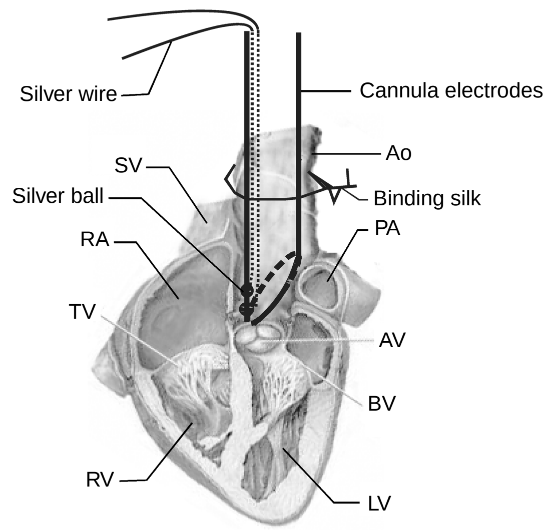


Fig. 2. Anatomic location of the cannula electrodes inserted site. Cannula electrodes were inserted into the aortic root and then fixed by a silk thread to bind with ascending aorta. The cannula electrodes are used for heart perfusion as well as HBE recording. Ao, aorta; AV, aortic valve; BV, bicuspid valve; LV, left ventricle; PA, pulmonary artery; TV, tricuspid valve; RV, right ventricle; RA, right atrium; SV, superior vena.

The cannula electrodes were inserted into the aortic root near the right aortic inner wall junction with right atrium (Fig. 2). The cannula electrodes were adjusted such that the H wave appeared between A (atrial depolarization) and V (ventricular depolarization) wave in HBE, and was then fixed by a silk thread so that the cannula electrodes was bound with ascending aorta.

Two silver wire electrodes were placed on the aorta and ventricular apex, respectively, to record epicardium ECG simultaneously. High right atrial pacing electrodes were positioned near the junction of the superior vena cava and right atrium. A pacing stimulus of 1-ms duration with an intensity of twice of the threshold current was generated by the inner-installed stimulator of the RM6240BD recording system and delivered to the heart preparation. Electrophysiological studies were performed according to standard methods described previously (1, 2). The right atrium was paced at a constant rate that is slightly faster than the spontaneous heart rate.

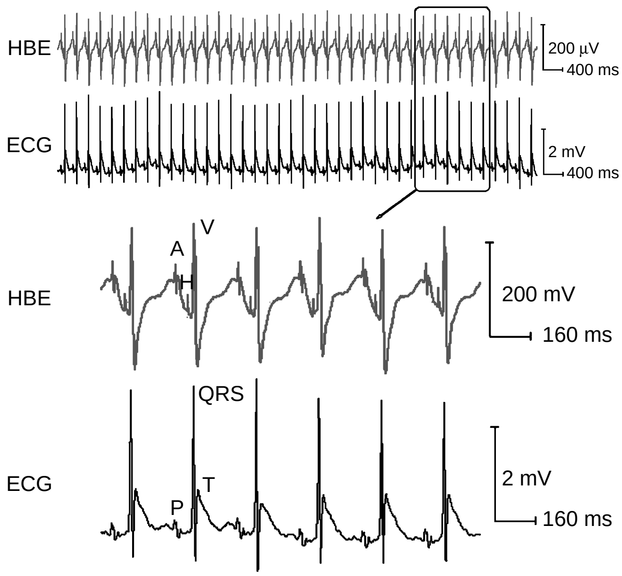


Fig. 3. Representative HBE and epicardium ECG of isolated rat heart. A, atrial depolarization; H, His bundle depolarization; V, ventricular depolarization.

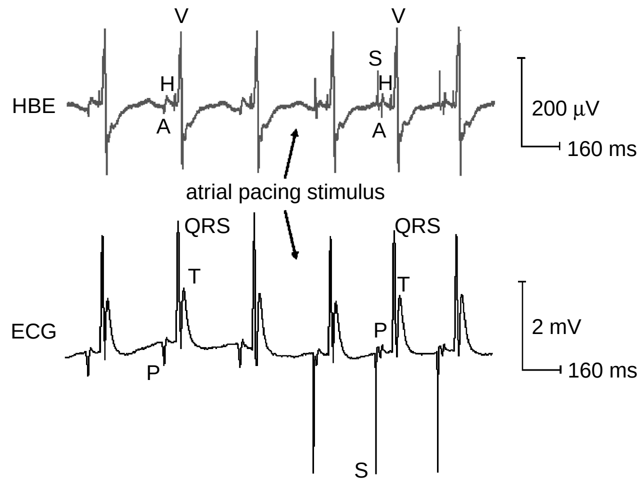


Fig. 4. Representative HBE and epicardium ECG under atrial pacing stimulus in the isolated rat heart. The right atrium near the superior vena cava was paced at a constant rate with a pacing cycle length of 250 ms. A, atrial depolarization; H, His bundle depolarization; V, ventricular depolarization; S, stimulation artifact.

After a train of eight stimuli ($S_1$) of constant rate atrial pacing, a single premature stimulus ($S_2$) was introduced. The coupling interval ($S_1S_2$) between the last $S_1$ and the test stimulus ($S_2$) was progressively shortened in 5-ms steps after every train of stimuli until $S_2$ did not evoke an atrial depolarization wave $A_2$.

## Results

*HBE and Epicardium ECG*

The HBE and epicardium ECG of isolated rat heart was simultaneously recorded, and a typical example was presented in Fig. 3. The H wave is very clear. Electrophysiological recording and measurements were performed in ten rats for four hours. The His bundle signal recorded with our new designed electrodes was so stable that the activity was neither lost nor decreased in amplitude. The cardiac cycle length is 284 ± 11 ms, atrio-His bundle conduction interval (A-H) is 40 ± 2 ms and His-ventricular conduction interval (H-V) is 16 ± 1 ms (means ± s.e.m., n = 10).

*Determination of Refractory Period of Cardiac Conduction System*

After programmed atrial pacing stimulus was given, H wave is still very clear and stable (Fig. 4). The following data were obtained: Atrial effective refractory period (AERP) was the longest $S_1S_2$ interval that did not evoke an atrial depolarization wave $A_2$. AV nodal effective refractory period (AVNERP) was the longest $S_1S_2$ interval in which the evoked $A_2$ failed to evoke a His bundle depolarization wave $H_2$. The shortest conducted $V_1V_2$ interval was defined as the His-Purkinje functional refractory period (HPFRP). AERP is 46 ± 3 ms, AVNERP is 126 ± 6 ms and HPFRP is 145 ± 9 ms.

## Discussion

The HBE of rat heart is difficultly recorded due to the small heart, its quick beating and the small His bundle potential. A previously described method related to positioning recording electrodes in the AV valve plane of epicardiac surface (4) requires not only the signal averaging techniques, but also the expensive and sophisticated equipments due to the small amplitude of the His signal. This status limits its applications. Some invasive electrophysiological recording techniques, such as placing a unipolar electrode on the endocardium near the apex of the triangle of Koch following an atriotomy (1) or in the AV nodal area following a ventriculotomy, were then introduced (3). However, these methods are complicated in operation and the integrity of heart structure might have been destroyed in the process.

Zhang *et al*. (5) designed cannula electrodes combined with the functions of Langendorff-perfusion aortic cannula and recording electrodes. However, the cannula electrodes were only used to record the HBE of big animals such as adult rabbits or puppies. In their two-in-one electrodes, the recording electrodes are nomadic in the cannula which touch the myocardial tissue untightly, thus the recording of HBE is unstable and the amplitude of H wave is relatively small. In the present study, we modified their electrodes and developed new cannula electrodes by fixing the recording electrodes out of the cannula. After modification, the contact between electrodes and the myocardial tissue was improved and the electrodes were placed easily and tightly. After performing intubation to perfuse heart, only a little of adjustment is needed. We also observed that the requirement of ours was less critical than that of atriotomy electrodes on the precise placement of electrodes. Moreover, the heart structure remained intact. Specifically, it can be applied to the recording of the His bundle signal in the small animals. It can also be used to record the HBE in bigger animal heart, such as adult rabbits or puppies, if the cannula electrodes are made of the tube with a greater outside diameter.

The stable HBE was observed not only during running baseline control, but also during its response to stimulus delivery to the isolated rat hearts. Therefore, we believe that this kind of cannula electrodes, which is relatively reliable, inexpensive and easily producible, will be a useful tool for physiological and pharmacological research in cardiac function.

## Acknowledgments

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