The Isolated and Cross-circulated AV Node Preparation of the Dog

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HASHIMOTO, K., IJIMA, T., HASHIMOTO, K. and TAIRA, N. *The Isolated and Cross-circulated AV Node Preparation of the Dog*. Tohoku J. exp. Med., 1972, 107 (3), 263-275 — The isolated AV node preparation of the dog consists of the right atrium and ventricular septum, and thus includes the main conducting pathways from the sinoatrial node through peripheral Purkinje fibers. The preparation is cross-circulated with a donor dog through the cannulated right coronary, anterior septal (ASA), and posterior septal arteries (PSA). The preparation permits close-arterial injection of drugs into the AV nodal area selectively *via* either the PSA or the ASA. The preparation also permits direct visual placement of recording electrodes at desired sites along the main conducting system. The preparation runs for 12 hr or more. Relation of AV conduction to rates of atrial pacing observed in this preparation is essentially the same as that observed in the isolated hearts. Occlusion of the PSA causes impairment of AV conduction, while that of the ASA causes only diminution of ventricular potentials. Acetylcholine causes a negative dromotropic effect and *l*-norepinephrine a positive one *via* both the PSA and the ASA. However, the two substances are more effective *via* the PSA than *via* the ASA. — AV conduction; acetylcholine; norepinephrine

Since atrioventricular (AV) conduction is one of common interests in the medical science, a considerable amount of information is available concerning effects of various drugs on AV conduction. However, AV conduction of the heart *in situ* is variously affected by many extracardiac factors, and results obtained from such preparation are not so simple to explain the mode of action of drug on AV conduction. Thus, elaborate pioneer works were performed by using the heart-lung preparation (Kramer *et al.* 1951) or the Langendorff preparation (Alanís *et al.* 1958). On the other hand, the recent development of the technique for the selective perfusion of the AV nodal area of the dog heart *in situ* through the posterior septal ("AV node") artery provided entirely new approach for the investigation of the mode of drug action of the AV nodal area (Nadeau and Amir-Jahed, 1965). This facilitated gain on physiological and pharmacological knowledges of the AV nodal area (Chiba *et al.* 1969a, b, Gauthier and Nadeau 1969, Chiba and Hashimoto 1970). However, for cannulation of the "AV node" artery, the posterior descending artery distal to the origin of the former must be ligated.

Received for publication, December 28, 1971.

This results in ischemia in the posterior part of the ventricular myocardium, larger or smaller in its extent. Recently two of us (Hashimoto and Hashimoto 1971, 1972) perfused *in situ* the anterior septal artery which supplies the ventricular septum without producing the myocardial ischemia.

We prepared the isolated AV node preparation of the dog heart. The preparation was essentially composed of the right atrium and the ventricular septum, and thus included the main conducting pathways from the sinoatrial node through peripheral Purkinje fibers. The preparation was cross-circulated with a donor dog through the cannulated right coronary, "AV node", and anterior septal arteries. In this paper the technique for making the preparation and the fundamental characteristics of its AV conduction are described.

METHODS

Atrioventricular (AV) node preparations

Adult mongrel dogs weighing 6–8 kg were anesthetized with ether and exsanguinated from the cut right carotid artery after an i.v. injection of 200 U/kg of sodium heparin. After 300–400 ml of blood were shed, the thorax was opened at the fifth right intercostal space and the heart was quickly removed. The excised heart was immediately immersed in Tyrode solution at about 4°C and gassed with a mixture of 95% O₂ and 5% CO₂. In this circumstance the heart ceased the spontaneous beats. In most hearts the anterior septal artery (ASA) which supplies the ventricular septum submerged immediately into the myocardium after it branched off the anterior descending artery (ADA). Thus, cannulation of the ASA was performed as described previously by the two of us (Hashimoto and Hashimoto, 1971). The origin of the ASA was dissected free and a fine cotton ligature was placed at its origin. Then, the initial segment, 2–3 mm, of the ADA was dissected free and a second ligature was tied. An incision of the artery was made proximally to the second ligature. A 13-gauge polyethylene cannula tapered down to about less than 1 mm in diameter at the tip and filled with Tyrode solution was inserted into the ASA through the incision made on the ADA. The first and second ligatures were tied to fix the cannula. Since the posterior septal artery (PSA) which supplies the atrial margin of the AV node (James 1964, Nadeau and Amir-Jahed 1965) was usually too thin to permit direct cannulation, the PSA was cannulated indirectly through the left circumflex artery (LCA) of which branch the PSA is. The initial segment of the LCA, about 10 mm, was dissected free and a polyethylene cannula similar to that to the ASA was inserted and fixed. The posterior descending artery, a main branch of the LCA, and other branches supplying the left atrium and the left ventricular free wall were ligated leaving the PSA alone intact. Since preliminary experiments showed that the right atrium and consequently the ventricular septum became unresponsive to electrical stimulation delivered at the crista terminal region unless the right coronary artery (RCA) was perfused with blood, the RCA was cannulated for perfusion. After the initial segment of the RCA, about 10 mm, was dissected free and cannulated, its branches supplying the right ventricular free wall were all ligated. Then, the left atrium and the right and left ventricular free walls were removed leaving the ventricular septum. The right atrium was incised to expose the right aspect of the interatrial septum and the endocardial surface of the right atrium. Fig. 1 shows the accomplished AV node preparation with three cannulae in the RCA, ASA and PSA inserted.

The three polyethylene cannulae were connected with rubber tubing, each of which was joined together to a piece of polyethylene tubing. Arterial blood led from the cannulated carotid artery of a donor dog was made to flow into this tubing through an electromagnetic flow probe (Nihon Kohden, MF-2T) by means of a peristaltic pump (Harvard Apparatus, Model 600–1200). A pneumatic resistance was set in parallel with the perfusion circuit

Fig. 1. The AV node preparation drawn schematically. The right coronary artery (RCA) and the anterior septal artery (ASA) are cannulated. The posterior septal artery (PSA) is cannulated *via* the left circumflex artery (LCA). ADA: the anterior descending artery; Ao: the aorta; AVN: the atrioventricular node; CS: the coronary sinus; CT: the crista terminalis; IVC: the orifice of the inferior vena cava; RBB: the right bundle branch; PA: the orifice of the pulmonary artery; PM: the anterior papillary muscle of the right ventricle; SVC: the orifice of the superior vena cava.

Fig. 2. Diagram of cross-circulation of the AV node preparation of the dog.

so as to maintain the blood flow at a constant pressure of 120 mm Hg. Total flow rate through the three cannulae was continuously monitored. Flow rate through each cannula was measured as reduction from the total flow rate when each rubber tubing was clamped for a brief period. Schematic diagram of cross-circulation of the AV node preparation is depicted in Fig. 2.

The preparation was placed in a funnel-shaped glass jacket through which water at about 40°C was circulated. The organ jacket was covered with a Lucite lid, and thus the surface of the preparation was kept moist and at 37–38°C. Venous blood from the preparation was collected in the bottom of the jacket, led to a venous reservoir, and, in turn, returned to the donor dog *via* the cannulated jugular vein.

As donors, mongrel dogs of both sexes, weighing 18–30 kg, were anesthetized initially with an i.v. injection of 30 mg/kg of sodium pentobarbital. A constant level of anesthesia was maintained by supplementing 2–2.5 mg/kg of the same anesthetic every half an hour. The animals were heparinized with an initial dose of 250 U/kg of sodium heparin and supplemental ones of 100 U/kg every half an hour. Respiration of the animals was aided with a dog respirator (Harvard Apparatus, Model 607). The systemic blood pressure was monitored at the femoral artery by means of an electromanometer (Nihon Kohden, MP-2).

Stimulating and recording device

Stimulating electrodes consisted of two pieces of ball-tipped silver-silver chloride wire embedded in a small Lucite plaque keeping an interelectrode distance about 2 mm. They were attached with fine silk sutures onto the endocardial surface of the crista terminal region. Rectangular pulses of 1-msec duration and voltage of two or three times the threshold voltage generated by an electronic stimulator assembly (Nihon Kohden, MSE-40) were delivered through an isolation unit (Grass, SIU4A).

Recording electrodes were also an assembly similar to a stimulating one but consisted of three electrodes keeping interelectrode distances about 2 mm each other. The atrial recording electrodes were attached onto the endocardium of the free wall of the right atrium about 10 mm from the coronary sinus with fine silk sutures. The ventricular electrodes were located at the endocardium about 5 mm from the base of the anterior papillary muscle of the right ventricle toward the AV ring. This location usually permitted simultaneous recording of the electrical activity of the right bundle branch and that of the underlying myocardium. By monitoring visually action potentials on the screen of a monitor cathode-ray oscilloscope (Nihon Kohden, VC-7), an appropriate pair of electrodes in each assembly were selected to pick up action potentials as simple and spike-like deflections.

The electrical activities thus picked up were displayed conventionally on the screen of a cathode-ray oscilloscope (Nihon Kohden, VC-6) and finally recorded on X-ray film by means of a long-recording camera (Nihon Kohden, PC-1B). In most sorts of experiment, however, the shape of the action potentials of the right atrium, right bundle branch and ventricular septum remained practically unchanged. Therefore, we used an AV interval-graph (Taira *et al.* 1971) for saving a laborious work to measure the conduction time. In addition, this device permitted us to observe continuously changes in the conduction time. Principle of the device is as follows: The action potential from the atrial electrodes and those of the right bundle branch and the underlying ventricular septum from the ventricular electrodes were amplified with two RC amplifiers with a time constant of 0.5 or 1 msec for a rising phase of each potential to be transformed to a pulse of short duration. After the outputs of the two RC amplifiers were mixed, each pulse was reshaped to a rectangular pulse of 0.5- to 1-msec duration and of constant amplitude by a monostable circuit. The rectangular pulses were fed as brightening pulses to the Z-axis of one channel of a dual-beam cathode-ray oscilloscope (Nihon Kohden, VC-6). The beam of this channel was swept synchronously with each stimulus pulse and its intensity was adjusted so that bright spots emerged on the oscilloscope screen only when the brightening pulses were fed to the Z-axis. With this setup in each sweep, after the first spot corresponding to a stimulus artefact, spots corresponding to the rising phases of potentials of the right atrium, right bundle branch and ventricular septum emerged in succession on the oscilloscope screen with a suitable latency or conduction time. Fig. 3 shows photographic records obtained by using one beam of the oscilloscope to display either the atrial potential or the potentials of the right bundle branch and the ventricle in the conventional configuration, and the other beam as the AV interval-graph.

Acetylcholine (ACh) chloride and *l*-norepinephrine (*l*-NE) base were used. ACh chloride was dissolved in 0.9% saline and *l*-NE base in 0.01 N HCl to give a concentration of 1 μ mol/ml. ACh and *l*-NE solutions thus made up were kept as stock solutions. Just prior to the experiment a series of concentrations were made by diluting the stock solutions

Fig. 3. Photographic records showing temporal relation of AV interval-grams to original potentials picked up bipolarly from the right atrium, the right bundle branch, and the ventricular septum. Rising phases of stimulus artefacts (S), the potentials of the right atrium (A), of the right bundle branch (B), and of the ventricular septum (V) are converted by an AV interval-graph to dots S', A', B', and V'. Intervals A'B', B'V', and A'V' are the time required by an impulse to travel from the right atrium to the right bundle branch, from the right bundle branch to the myocardium of the ventricular septum, and from the right atrium to the ventricular septum respectively.

with 0.9% saline. Injected volumes measured constantly $30\ \mu\text{l}$ and were injected at a constant rate for 4 sec into the rubber tubing proximal to the atrial cannula with $50\text{-}\mu\text{l}$ microsyringes (Jintan Terumo)

RESULTS

Recovery process of rhythm and blood flow

Soon after cross-circulation started, the quiescent preparation regained its activity; the atrium began to beat spontaneously and the ventricular septum showed fibrillatory contractions. In 30 to 40 min most preparations resumed normal AV conduction. From this time on the preparations ran for 12 hours or more within which the scheduled experiment usually was finished.

Just after the commencement of cross-circulation total blood flow through the three cannulated arteries amounted up to 40 to 60 ml/min under a perfusion pressure clamped at 120 mm Hg. However, until normal AV conduction resumed, blood flow decreased gradually to a certain value and remained little changed for 12 hours or more. In this state the flow rate through the PSA was about 7 ml/min, that through the ASA about 8 ml/min and that through the RCA about 5 ml/min. When blood flow through the PSA decreased, AV conduction was readily impaired. Therefore, the effect of arterial occlusion on AV conduction was investigated.

Effect of occlusion of the PSA and of the ASA on AV conduction

In these experiments atrial and ventricular potentials alone were recorded. At a fixed stimulus interval of 400 msec the AV conduction time was in the range of 110 to 130 msec and remained constant for a given preparation. Arterial occlusion was achieved simply by clamping with a pair of hemostats the rubber blood-conducting tubing just proximal to the arterial cannula. In all of five preparations occlusion of the PSA for 2.5 to 3 min caused impairment of AV conduction, although the severity of impairment depended on preparations. Upon occlusion the AV conduction time was little changed for the first one minute. However, in two of the five preparations for the following 1 to 1.5 min the AV conduction time was progressively prolonged by up to 50 to 60 msec and finally AV block of Wenckebach type and 2:1 block took place. Even at this stage the configuration of ventricular potentials were not changed at all. In the remaining three preparations the AV conduction time was prolonged only by 10 to 30 msec. Upon release of occlusion AV conduction recovered within about 1.5 min. An example of such five experiments is depicted in the left panel of Fig. 4 in which

Fig. 4. Effects on AV conduction of occlusion of the posterior septal artery (PSA) and that of the anterior septal artery (ASA). The right atrium was driven at an interval of 400 msec. In each pair the upper records show the atrial and the lower ones the ventricular potentials. Each record is formed by superimposition of 12-13 traces. Periods of arterial occlusion are indicated by vertical bars. In this preparation occlusion of the PSA for 2.5 min caused prolongation of the AV conduction time, while that of the ASA for 5 min caused diminution of the ventricular potentials without significant prolongation of the AV conduction time.

traces of atrial lead (upper traces of each pair) and those of ventricular lead (lower traces of each pair) were superimposed for 2 sec every 15 sec.

Occlusion of the ASA for 3 to 5 min, unlike that of the PSA, caused diminution of amplitude of the ventricular potentials for the late 2 min in two of the four preparations. Even in this stage the AV conduction time was not changed substantially. Upon release of occlusion the ventricular potentials regained their control amplitude within about 30 sec. The right panel of Fig. 4 shows such an example obtained in the same way and in the same preparation that is shown in the left panel. In the remaining two preparations neither amplitude of the ventricular potential nor the AV conduction time was changed at all.

Relationship between stimulus intervals and AV conduction

Relationship between stimulus intervals and the AV conduction time as measured in 10 preparations was presented in Table 1. Since the sinoatrial node was left intact, the sinus rhythm took over the driving rate when stimulus intervals

TABLE 1. *Relation of AV conduction time to stimulus intervals of atrial pacing*

Prep. No.	AV conduction time (msec) at stimulus interval (msec) of			
	500	400	320	250
1	106	108	110	WB*
2	126 (112)	129 (116)	137 (124)	—
3	128 (120)	129 (121)	136 (128)	WB
4	115	115	118	WB
5	106 (96)	110 (100)	120 (110)	WB
6	114 (92)	119 (95)	126 (100)	WB
7	120 (96)	125 (100)	134 (110)	2:1 block
8	118	118	122	WB
9	120 (90)	122 (92)	139 (109)	3:1 block
10	120 (114)	122 (116)	126 (120)	WB

Time required by an impulse to travel from the right atrium to the right bundle branch is shown in parentheses.

* AV block of Wenckebach type.

were longer than 400 or 500 msec. As can be seen in this table, a marked increase in AV conduction time occurred at stimulus intervals between 400 and 320 msec. At a stimulus interval of 250 msec various types of AV block took place.

Effects on AV conduction of ACh injected into the PSA and the ASA

In accord with results in the *in situ* AV node preparations (Nadeau and Amir-Jahed 1965, Hashimoto and Hashimoto 1971, 1972), when injected into the PSA, ACh caused a transient prolongation of the AV conduction time in doses of 0.01 to 1 nmol and complete AV block in doses of 0.1 to 10 nmol. In these circumstances the configuration of the ventricular potential was not changed at all. In general, the shorter the stimulus interval, the more effective ACh in depressing AV conduction. Fig. 5 shows such an example in which intervals between trains

of dots denoted as A and those of V present the AV conduction time.

Close-arterial injection of ACh into the ASA also caused a transient prolongation of the AV conduction time or AV block without change in the ventricular potential shape. When compared at the stimulus interval of 400 msec, however, doses of ACh required to match the negative dromotropic effect caused by ACh injected into the PSA were about 30 times as much as those injected into the PSA. Fig. 6 shows such an example obtained in the same preparation that was used in

Fig. 5. AV interval-grams showing effect on AV conduction of acetylcholine (ACh) injected selectively into the PSA. Stimulus intervals (SI) of atrial pacing were 400 msec in upper panel and 320 msec in lower one. S: stimulus artefacts of atrial pacing. Dots denoted as A, B, and V present the time of arrival of impulses at recording sites of the right atrium, the right bundle branch, and the ventricular septum. Impairment of AB conduction by acetylcholine was pronounced at shorter stimulus intervals.

Fig. 5. It must be also noted that ACh injected into the ASA caused the positive dromotropic effect after AV block. When injected into the PSA, comparable doses of ACh scarcely caused a positive dromotropic effect after AV block.

Effects on AV conduction of l-NE injected into the PSA and the ASA

Close-arterial injection of *l*-NE in doses of 0.03 to 0.3 nmol into the PSA caused a dose-dependent decrease in the AV conduction time when the atrium was paced at an interval of 400 msec. However, there occurred no change in the configuration of the ventricular potential. With doses larger than 0.1 to 1 nmol the rhythm produced by the enhanced automaticity of the AV node took over the paced rhythm. In most preparations the AV nodal tachycardia took place at a time when the positive dromotropic effect reached its maximum. The left panel of Fig. 7 shows such an example. Despite uninterrupted atrial pacing disappearance of atrial potentials (dots denoted as A) indicates that the atrium was made

Fig. 6. AV interval-grams showing effect on AV conduction of acetylcholine (ACh) injected selectively into the ASA. Notations are the same as in Fig. 5. The negative dromotropic effect of acetylcholine was less dependent on stimulus intervals of atrial pacing.

refractory to atrial pacing by preceding impulses which invaded the atrium retrogradely from the AV node. The automaticity of the AV node enhanced by *l*-NE was readily suppressed by decreasing stimulus intervals (right panel of Fig. 7). The AV conduction time was longer at a stimulus interval of 320 msec than at 400 msec. However, with each dose of *l*-NE the AV conduction time became shorter at a stimulus interval of 320 msec than at 400 msec. In other words, the positive dromotropic effect of *l*-NE was more marked with pacing at 320 than at 400 msec.

Close-arterial injection of *l*-NE into the ASA also caused a positive dromotropic effect but no change in the ventricular potential shape. As with ACh, doses of *l*-NE required to match the effect caused by *l*-NE injected into the PSA were about 30 times those administered into the PSA. Even with increasing doses up to 30 nmol the AV nodal tachycardia was not produced but ventricular rhythm often took place.

Behavior of potentials of the right bundle branch and of the bundle-ventricular (BV) conduction time

When the placement of the ventricular recording electrodes was appropriate, the spike-like deflection of 20 to 30 mV signaling excitation of the ventricular septum at the recording site was preceded by a spiky deflection of a few millivolts at intervals of 8 to 14 msec (Fig. 3). These intervals varied from preparation to preparation in that range. But for a given preparation the interval remained

Fig. 7. AV interval-grams showing effect on AV conduction of *l*-norepinephrine (NE) injected selectively into the PSA. *l*-Norepinephrine caused a dose-dependent decrease in the AB conduction time, and consequently that of the AV conduction time. At a stimulus interval of 400 msec 1 nmol of *l*-norepinephrine caused so-called AV nodal tachycardia of which cycle length was shorter than 400 msec but longer than 320 msec.

Fig. 8. AV interval-grams showing the positive dromotropic effect of *l*-norepinephrine injected into the ASA.

constant. Judging from the location of the recording electrodes in reference to anatomical landmarks and comparing the configuration and temporal relation of the potential to the atrial and ventricular potentials with those obtained by previous workers (Burchell *et al.* 1953, Hoffman *et al.* 1959, 1960), the small spiky deflection is considered to be the electrical activity of the right bundle branch.

With alteration of stimulus intervals, the time required by an impulse to travel from the atrium to the right bundle branch (AB interval) was changed in parallel with the AV interval. But, the time required by an impulse to travel from the right bundle branch to the ventricular septum (BV interval) was not changed significantly (Table 1).

Upon injection of ACh and *l*-NE into the PSA the bundle potential behaved exactly like the ventricular potential. As can be seen in Figs. 5 and 7, AB intervals changed in parallel with AV intervals, while BV intervals remained unchanged.

When ACh and *l*-NE were injected into the ASA, AB intervals changed in parallel with AV intervals as was the case of injection into the PSA (Figs. 6 and 8).

DISCUSSION

In the present preparation values obtained as the AV conduction time with pacing the right atrium at stimulus intervals of 500 and 400 msec approximated those obtained in the dog heart-lung preparation (Krayner *et al.* 1951) and in the dog Langendorff preparation (Alanís *et al.* 1958). Furthermore, the relation of the AV conduction time to stimulus intervals observed in the present preparation also resembled that observed in the dog Langendorff preparation (Alanís *et al.* 1958). Thus, it is not unreasonable to consider that AV conduction of the present preparation represents normal conduction in the state free from the tonic neural influence. The preparation ran for 12 hours or more with such normal AV conduction.

The present preparation permitted us selective administration of drugs into either the RCA, the ASA, or the PSA. Furthermore, the preparation permitted us the direct visual placement of recording electrodes at any position along the conducting pathways, as the Langendorff preparation (Alanís *et al.* 1958) or the dog on cardio-pulmonary bypass (Hoffman *et al.* 1959, 1960) did. The simultaneous recording of the electrical activities both from the right bundle branch and from the underlying myocardium, for example, revealed the differential effects on AB and BV conduction of ACh or *l*-NE given into the PSA and of ACh or *l*-NE given into the ASA. Thus, the systematic location of recording electrodes in combination with the selective administration of drugs will facilitate more detailed analysis of the AV node activity.

Occlusion of the PSA and the ASA exerted differential effects on AV conduction in the present preparation. Occlusion of the PSA caused impairment of AV conduction ranging from simple prolongation of the AV conduction time to Wenckebach block or 2:1 block. This indicates that the structures directly concern-

ing major AV delay are nourished predominantly by the PSA, although the AV nodal area has been reported to receive dual supply from the PSA and ASA (Lumb *et al.* 1959, James 1964). In this respect the present results appear in disaccord with the results obtained by Nadeau and Amir-Jahed (1965) in the *in situ* AV node preparation that occlusion of the PSA caused no change in the PR interval. It is presumable that more anastomoses exist between the PSA and ASA in the *in situ* heart than in the excised AV node preparation. Lumb *et al.* (1959) reported that the ligation of the ASA caused severer impairment of cardiac function than that of the PSA. As a matter of fact, in the present experiments the amplitude of the potentials of the ventricular septum was greatly reduced upon occlusion of the ASA, although the AV conduction time was not prolonged substantially. If occurred in the *in situ* heart, cease of excitation of the upper septum might lead to the fatal cardiac dysfunction. In this regard the present results are not so different from those of Lumb *et al.* (1959).

In accord with the results obtained by arterial occlusion, both ACh and *l*-NE injected into the PSA was more effective in modifying AV conduction than those given into the ASA. This is the confirmation of the results obtained by the two of us (Hashimoto and Hashimoto 1971, 1972) with selective perfusion of the PSA and the ASA *in situ*. However, it must be noted that there are some qualitative differences in their effects depending on the route of administration. Even with increasing doses, *l*-NE given *via* the ASA failed to cause the AV nodal tachycardia, and ACh given *via* the PSA scarcely caused a positive dromotropic effect after AV block, as was observed on injection into the ASA. This reflects differences in structures which drugs reach *via* the two routes, as the anatomy of blood supply to the AV nodal area by the two arteries tells (Lumb *et al.* 1959, James 1964); the PSA supplies the AV junctional area and the upper part of the AV node, while the ASA the lower.

Acknowledgment

The present study was aided by grants from the Pharmacological Research Foundation, Inc., Tokyo, and Tokyo Research Laboratory, Kowa Company.

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