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The influence of the atrial myocardium on impulse formation in the rabbit sinus node

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Abstract. In the isolated right atrium of the rabbit heart the influence of the atrial myocardium on impulse formation in the sinus node was investigated. Under normal conditions the pacemaker (earliest activation) was located in the center of the node where fibers with the highest rate of diastolic depolarization were found. After disconnection of the atrium from the sinus node spontaneous cycle length decreased from a mean of 348 ms to a mean of 288 ms (−18%) in all experiments ($n = 15$). This was accompanied by a shift of the pacemaker from the nodal center towards the border zone. By means of multiple microelectrode impalements changes in action potential configuration were studied. After disconnection of atrium and sinus node the rate of diastolic depolarization of fibers in the border zone was increased from a mean of 26 mV/s to a mean of 78 mV/s, whereas in the center of the sinus node no increase was found (mean: 52 mV/s). It was concluded that the fibers in the border zone of the sinus node are better pacemaker fibers than in the nodal center. However under normal conditions the intrinsic pacemaker properties of the border zone fibers are electronically depressed by the connected atrial myocardium.

Key words: Sinus node – Impulse formation – Diastolic depolarization – Electrotonic influence – Sinus arrhythmia

Introduction

From electrophysiological and morphological studies it is clear that the rabbit sinus node is an inhomogeneous structure. Within the sinus node a nodal center and a transitional zone or border zone can be distinguished [2, 23, 31]. The border zone forms the connection between the nodal center and the atrium (crista terminalis). Normally the location of impulse origin in the isolated sinus node is found in the center [2, 4, 27]. However, it is not fixed and can shift to different sites within the node, accompanied by changes in rate of impulse formation. This indicates that generation of impulses is not reserved to one particular group of nodal cells, but can be taken over by other cell groups in the node.

Recent studies with computer models, cultured cell aggregates and sinus node specimen, indicated firmly that electrically coupled pacemaker fibers or fiber groups can modulate each others rhythmicity electrotonically [9–11, 17]. The amount of mutual electrotonic influence is related to the amount of coupling resistance [13, 21, 35]. The role of electrotonic interactions with respect to the electrophysiological behavior of the isolated rabbit sinus node was emphasized by several investigators [1, 3, 22, 23, 28].

However, beside the electrical coupling of fibers within the sinus node itself, this structure is also electrically coupled to the relatively large atrium. It was the aim of the present study to investigate the influence of this atrium on impulse formation in the intact sinus node.

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Methods

Rabbits of either sex weighing 1.5–2.0 kg were killed by cervical dislocation. The thorax was opened by midsternal thoracotomy. The pericardium was opened and the heart was excised quickly. The preparation including the right atrial appendage, crista terminalis and the intercaval tissue with the sinus node, was made while immersed in oxygenated Tyrode's solution (temperature 25–30° C). Then the preparation was pinned upon a silicon bar, placed in a tissue bath (35 ml) with its endocardial side facing upward. The tissue bath was continuously refreshed by oxygenated Tyrode's solution (100 ml/min) at a temperature of 37° ± 0.2° C. The Tyrode's solution contained 130 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 0.6 mM $MgCl_2$, 24.2 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 11 mM glucose and 13 mM sucrose. It was saturated with 95% O_2 and 5% CO_2. The pH was 7.35 ± 0.05.

After finishing the preparation no measurements – except continuous registration of an atrial surface electrogram – were performed during at least 30 min until sinus rhythm was completely stable. Then location of impulse formation and nodal activation pattern were determined both under control conditions and after disconnection of the atrium from the sinus node. Action potential configuration before and after disconnection were studied as well.

Experimental protocol. Under control conditions a surface electrogram, recorded with a bipolar electrode (teflon-coated silver wire, 0.25 mm diameter, 0.3 mm interpolar distance) placed upon the crista terminalis, was monitored continuously. While using standard microelectrode techniques (glass capillaries filled with 3 M KCl and a tip resistance of 15–30 MΩ) multiple impalements (80–120; at 300 μm distance) in the sinus node region were made. The moment of activation of every impaled fiber was determined

by calculation of the time difference between the action potential upstroke (50% level of the amplitude) and the intrinsic deflection of the surface electrogram. The site where fibers were activated earliest was interpreted as the location of impulse origin. This site was designated as time reference zero. Propagation of the impulse through the sinus node was determined by calculation of the activation latency between this zero time reference and the upstroke of fibers in other locations of the preparation. In this way it was possible to determine the location of the pacemaker and the activation pattern of the sinus node. However, the described method is only valid if sinus rhythm remains constant and pacemaker location is fixed during the time required to construct an activation map (2–3 h). This was checked by continuous measurement of beat to beat interval and consideration of the shape of the atrial electrogram. Changes would suggest a shift of the location of the pacemaker, whereafter data were rejected. All recordings were stored on magnetic tape (Ampex PR 2200) for off-line analysis.

After finishing this part of the experimental protocol the transition between the crista terminalis and the border zone of the sinus node was determined optically (less reddish appearance of the nodal – border zone – fibers under a dissecting microscope) and by means of microelectrode impalements (typical action potential configuration of border zone fibers). Under a dissecting microscope (16 times magnification) the crista terminalis and the connecting atrial appendage were separated from the sinus node by making an incision with a fine pair of scissors along this sinoatrial transition. After this procedure the surface electrode was replaced at the edge of the nodal part of the preparation, and monitoring of spontaneous activity as well as calculation of the beat to beat interval was restarted immediately. After a stabilization period of at least 30 min location of the pacemaker and activation pattern of the sinus node were determined again following the above described method. The impalements were made at the same sites as before disconnection of atrium and sinus node. In this way it was possible to compare location of the pacemaker and activation pattern as well as action potential configurations of the sinus node with and without attached atrium.

Results

Spontaneous activity before and after disconnection of crista terminalis and sinus node

Separation of crista terminalis and sinus node was performed in 15 experiments. Under control conditions (before separation) the mean cycle length of these preparations was 348 ± 50 ms (mean ± SD) and completely stable. Two of these preparations had a relatively fast sinus rate (about 4 Hz; beat to beat interval 260 ms) and it turned out that in these cases the pacemaker was located in the border zone. Without these two preparations the mean cycle length was 361 ± 38 ms (mean ± SD). After determination of pacemaker location and activation pattern, the crista terminalis was disconnected from the sinus node following the above described procedure. In all preparations, except the two with the rapid rate mentioned above, sinus rate began to accelerate immediately after the disconnection. This period of acceleration lasted about 10–20 min. Subsequently cycle length stabilized and remained stable during the rest of the experiment which lasted at least 4 h. After about 1 h the mean cycle length of these preparations was 294 ± 42 ms (mean ± SD). This is a significant decrease of 18% ($p < 0.001$) in comparison to control situation. Figure 1 depicts schematically the changes in cycle length of every experiment. Notice the two preparations with a relatively fast spontaneous rate under control conditions (indicated by the dotted lines).

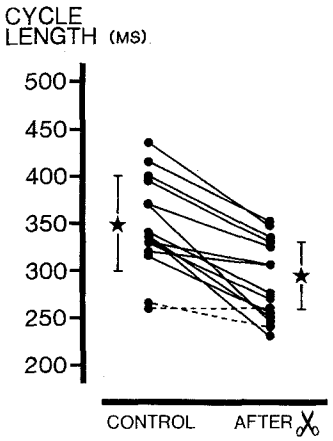

Fig. 1. Changes in spontaneous actvitiy (beat-to-beat interval in ms) of the sinus node after removal of the atrium. The findings of each experiment are given (●) as well as the mean values of sinus interval of all experiments ($n = 15$) are shown (★). Notice the two experiments in which the interval under control conditions is already short. The pacemaker was located in the border zone, and disconnection of the atrium has almost no effect on sinus rate

Changes in activation pattern after separation of atrium and sinus node

In the upper part of Fig. 2 the activation pattern of one of the preparations is depicted. The pacemaker (earliest activation) is located at a distance of about 0.7 mm from the crista terminalis, which is within the center of the sinus node. The activation pattern of the preparation is indicated by isochrones, showing the area which was activated within subsequent periods of 10 ms. The impulse is conducted very slowly in the center of the sinus node, but accelerates in cranial direction towards the border zone and finally reaches the crista terminalis within 20 ms. The activation pattern of the other 12 preparations was similar. In these experiments the mean distance of the pacemaker from the crista terminalis was 600 ± 124 µm and the mean activation moment of the crista terminalis was 21 ± 11 ms after impulse origin. Therefore the given example can be considered as representive.

In the lower part of the figure the situation (same preparation) after disconnection of the crista terminalis from the sinus node and stabilization of the accelerated rhythm is depicted. The pacemaker is shifted from its original site in the nodal center to a site in the border zone of the sinus node. During the further course of the experiment this location was maintained. However, this pacemaker shift caused a complete change of the activation sequence of the node. The impulse is conducted from the border zone towards the nodal center and thus in an opposite direction compared to the control situation. The described shift of the pacemaker towards a location in the border zone and the consequent changes in activation pattern were seen in all 13 prepara-

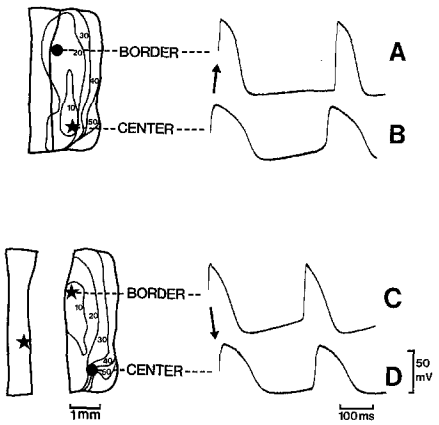

Fig. 2. Changes in action potential configuration, pacemaker location and activation pattern after disconnection of atrium and sinus node. The *upper part* of the figure depicts the control situation. Action potential configuration is conform the normal findings in the sinus node: earliest activation and steepest diastolic depolarization in the nodal center (tracing *B*), activation latency and slow rate of diastolic depolarization in the border zone (tracing *A*). The *lower part* of the figure shows that after disconnection of atrium and sinus node rate of diastolic depolarization in the border zone fiber (tracing *C*) is increased markedly. This was not the case in the center of the node (tracing *D*). The pacemaker is shifted from the nodal center towards the border zone. Activation sequence of the sinus node, visualized by means of isochrones (steps of 10 ms) has changed concommitantly

tions. However, there was no predelicted location within the border zone for the pacemaker to shift towards. In some experiments it was located cranially, in other experiments caudally.

Under control conditions in two experiments the pacemaker was located in the border zone of the sinus node accompanied by a nodal activation sequence from the border zone towards the nodal center. Removal of the atrium was in one case followed by a shift of the pacemaker to another location in the border zone and a 15 ms shortening of sinus interval whereas in the other case no changes were found.

Action potential configuration before and after disconnection of crista terminalis and sinus node

In Fig. 2 two (representative) pairs of action potentials recorded from fibers in the border zone and in the center of the sinus node are displayed. Sites of impalements correspond to the location of the pacemaker before (nodal center) and after (border zone) separation of atrium and sinus node. Under control conditions the action potential from the fiber in the center of the node (tracing B) shows typical pacemaker characteristics: earliest activation, a slow upstroke, a long action potential duration, a low maximal diastolic potential and a high rate of diastolic depolarization followed by a smooth transition to the upstroke of the action potential. The action potential recorded from a fiber in the border zone (tracing A) is characterized by a steep upstroke, a short action potential duration, a low rate of diastolic depolarization followed by an abrupt transition to the upstroke and a time latency with respect to the action potential recorded from the fiber at the site of earliest activation. However, removal of the atrium was followed by a shift of the pacemaker towards the border zone and a marked change in

Table 1. Changes in action potential configuration, $n = 5$

	Control	Isolated SA	Significance
Border zone			
RDD (mV/s)	26 ± 12	78 ± 16	***
MDP (mV)	−61 ± 6	−62 ± 6	n.s.
APD (ms)	59 ± 9	67 ± 9	***
APA (mV)	74 ± 13	67 ± 10	***
Center			
RDD (mV/s)	52 ± 19	50 ± 28	n.s.
MDP (mV)	−56 ± 11	−56 ± 10	n.s.
APD (ms)	67 ± 19	72 ± 11	***
APA (mV)	56 ± 12	51 ± 13	**

Statistics of different action potential parameters obtained from microelectrode impalements (10 – 20) at constant distances from the crista terminalis in 5 experiments.
APD: action potential duration at 50% level of the amplitude; MDP: maximal diastolic potential; RDD: rate of diastolic depolarization; APA: action potential amplitude; significance (paired): $*p < 0.05$; $**p < 0.01$; $***p < 0.001$

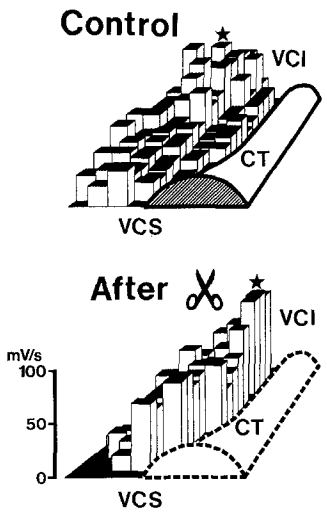

Fig. 3. Spatial distribution of diastolic depolarization rate before (*upper part*) and after (*lower part*) atrial disconnection. The ground plate represents the tissue sheet in which the sinus node is located. The amplitude of the *vertical bars* represents the rate of diastolic depolarization (mV/s) as found at the corresponding site. The *asterisk* indicates the site of earliest activation (pacemaker location). Notice that the preparation is turned over 180°, compared to the activation maps in Fig. 2, in order to get the best overview. The previous location of the crista terminalis is indicated by a *dotted line*. *VCS* vena cava superior; *VCI* vena cava inferior; *CT* crista terminalis

action potential configuration compared to the control situation: the rate of diastolic depolarization of the action potential recorded from the fiber in the border zone (tracing C) was increased markedly, whereas in the center of the node (tracing D) no increase and even a slight decrease in rate of diastolic depolarization was observed.

In each of five experiments a series of 10 – 20 impalements, arranged in a line parallel to the crista terminalis and at a distance of 200 μm as well as 800 μm from the crista terminalis were performed before and after disconnection of sinus node and atrium. The distance between impalements

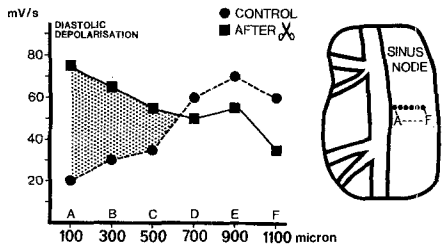

Fig. 4. Relation between distance from the atrium (crista terminalis) and rate of diastolic depolarization in the sinus node. At the *right* the location of the impalements within the preparation. The relation between rate of diastolic depolarization and distance from the atrium is more or less reversed after disconnection of the crista terminalis from the sinus node. The *shaded area* represents the electrotonic influence of the atrium on the pacemaker potential. Impalement *A* was made at a distance of about 100 μm from the crista terminalis, whereas the distance between the subsequent impalements was 200 μm

in a line was 300 μm. Different action potential parameters were determined before and after the disconnection and are given in Table 1.

Spatial distribution of diastolic depolarization rate in the sinus node

We measured the rate of diastolic depolarization at a large number of sites within the sinus node before and after removal of the atrium. The results of one of these experiments are depicted in Fig. 3. Related to the contours of the preparation, the rate of diastolic depolarization is represented by the amplitude of the vertical bars. In the upper part of the figure the control situation is depicted. As can be seen, the highest rates of diastolic depolarization are found in a distinct area in the caudal part of the preparation (at the side of the vena cava inferior), a region corresponding to the nodal center. Location of the pacemaker (black star) coincides with the site of steepest pacemaker potential. The distribution of rate of diastolic depolarization in the same sinus node but without attached atrium is given in the lower part of Fig. 3. It is obvious that the rate of diastolic depolarization in the border zone is increased markedly. However, in the center of the node no such increase can be observed. The highest rates of diastolic depolarization are found in the border zone which explains the shift of the pacemaker to this region.

This spatial distribution of diastolic depolarization rate was found in all experiments in which the pacemaker was under control conditions located in the nodal center. Therefore the above described preparation can be regarded as representive.

To investigate if the changes in diastolic depolarization rate within the sinus node are related to the distance from the crista terminalis, we made a series of impalements at increasing distances from this structure both before and after disconnection from the sinus node. Figure 4 depicts the results obtained from one of these experiments. Under control conditions diastolic depolarization rate in the border zone, at a distance of about 100 μm from the crista terminalis, was 20 mV/s. This increased at greater distances, especially at about 600 μm (between location C and D) which coincides roughly with the transition from border zone to nodal center. At about 900 μm (location E), in the nodal center, the maximal rate of diastolic depolarization was found and amounted to 70 mV/s. At 1100 μm the rate of diastolic depolarization was 60 mV/s and decreased further at locations more distant from the atrium (not shown in the diagram). Thus, under control conditions the rate of diastolic depolarization increases at greater distances from the atrium. However, after disconnection of crista terminalis and sinus node this relation was reversed. The maximal rate of diastolic depolarization was found in location A and was 75 mV/s. This is even higher than the maximum value found under control conditions. From this site rate of diastolic depolarization decreased gradually to location E (900 μm) where it was 57 mV/s, which is 13 mV/s less than under control conditions when the pacemaker was located in this region. At location F rate of diastolic depolarization was only 33 mV/s and decreased further at greater distances (not shown in the diagram).

Thus, the shaded area in the diagram in fact represents the depression of diastolic depolarization by the attached crista terminalis. Similar findings were obtained from other experiments. The crossing point of both graphs was always located at distances between 600 and 900 μm from the margin of the crista terminalis.

Discussion

To investigate the influence of the crista terminalis on impulse formation in the sinus node it was necessary to separate both parts of the preparation along a sharp line with minimal loss of nodal fibers. Therefore we used a small pair of scissors to make an incision.

Why does the rate of diastolic depolarization become elevated in fibers of the border zone after disconnection of sinus node and crista terminalis? Two possible explanations are available for this phenomenon. First, fibers in the border zone, injured by the pair of scissors, might be depolarized. This will increase the rate of diastolic depolarization and induce enhanced automaticity. Second, the rate of diastolic depolarization in the border zone fibers is under normal conditions depressed, due to current loss to the atrial myocardium.

With respect to the first explanation it is obvious that a number of fibers, located at both sides of the incision, will be damaged. However, several arguments make a significant role of injury very doubtfull. If the acceleration of the nodal pacemaker was due to increased automaticity of injured cells, one would expect this acceleration to disappear after a short period of time when the injured cells are definitely dead or "healed-over". This "healing-over" process lasts 15 min maximally, whereas the accelerated rhythm maintained during the further course of the experiment, lasting 3–4 h [12, 14, 16]. The action potential configuration after disconnection of atrium and sinus node was not significantly changed (with exception of the diastolic depolarization rate). If cells were injured one would expect them to be depolarized. This was not the case since no significant changes in maximum diastolic potential were found (Table 1). Another important indication that the injury by the incision did not induce rapid automaticity is given by the results of an earlier study. A similar incision parallel to the crista terminalis at a distance of about 500 μm was made to separate border zone and nodal center; it appeared that both parts of the preparation were spontaneously active

with about the same frequency as before the division (361 ± 29 ms before the incision and 348 ± 32 ms in the central nodal part and 357 ± 31 ms in the border zone after the division; $n = 25$) [5]. The incision obviously had not caused a rhythm acceleration.

The second explanation for the acceleration of spontaneous activity is based on the existance of electrotonic interactions between the atrial myocardium and the nodal fibers. The diastolic potential difference will cause a current flow through the intercellular connections between nodal and atrial fibers. This current has a polarizing effect on the nodal fibers and counteracts diastolic depolarization to some extent. On the other side this current will cause a depolarization of the atrial fibers. However, this probably is indetectable because of the enormous tissue mass of the crista terminalis in comparison to that of the sinus node.

It is obvious that the amount of current flow is dependent on intercellular coupling and distance. The present findings made evident that the electrotonic influence does not reach the nodal center. This is in accordance with findings of other investigators who determined the space constant in the sinus node (Bonke 1973: 500 μm; Seyama 1976: 800 μm; Bukauskas et al. 1977: 200–600 μm [3, 8, 29]. From recent studies it appeared that the distribution of intercellular connections within the sinus node is not homogeneous [18, 25]. In the center of the node the amount of intercellular coupling is less than in the border zone; consequently the space constant will decrease going from the border zone towards the nodal center and this will also diminish electrotonic influence from the atrium.

The amount of intercellular coupling in the cranial part of the sinus node is greater – and thus the atrial influence stronger – than in the caudal part [25]; this might explain the lower rate of diastolic depolarization in this region in comparison to the caudal part of the node, where the pacemaker is located (see Fig. 3).

Releasing the sinus node from the atrial myocardium decreases action potential amplitude and increases action potential duration in the sinus node. This can be expected since the crista terminalis fibers are activated only some milliseconds later than the border zone fibers. The amplitude of the atrial action potential is higher and thus current will flow which increases amplitude of action potentials of border zone fibers. The same happens during repolarization which starts earlier and is devoided of a plateau phase in atrial fibers (shorter action potential duration). The consequent current flow will shorten action potential duration of the border zone fibers. Based on electrotonic currents a decrease of the maximal diastolic potential is expected as well. However, this was not found in the present experiments. It should be realized that at the moment of maximal diastolic potential the electrotonic current flow between atrial and nodal fibers will be minimal and probably indetectable with the present method. A continuous intracellular recording in the border zone before, during and after the disconnection procedure would be necessary to answer this question. However, this was a technical impossibility.

The slight decrease in diastolic depolarization rate which was found in the nodal center after atrial disconnection might be caused by the overdrive suppressant effect of the accelerated border zone pacemaker.

From studies in the past several conditions are known which influence sinus rate and pacemaker location as well: vagal stimulation, administration of acetylcholine or (nor)epinephrine, temperature changes, electrolyte disturbances or drugs [6, 7, 24, 30, 33]. Changes in electrotonic interactions during these conditions might be the underlying mechanism. For example digitalis, which decreases intercellular coupling [15, 34], causes at certain concentrations a rhythm acceleration and shift of the pacemaker towards the border zone of the sinus node [30, 32].

The present results are in accordance of those of other investigators. In recent studies the sinus node was divided in pieces and it was found that the specimen originating from the peripheral region of the sinus node, usually indicated as latent pacemaker region, were also capable of spontaneous formation [23, 26]. However, the used methods eliminated electrotonic influences both of the atrium on the sinus node as well as within the sinus node itself. The present method exclusively investigates the electrotonic influence of the atrium.

What are the clinical implications of the present findings? When the coupling between atrial myocardium and sinus node fibers is decreased for some reason (ischemia, digitalis intoxication) sinus tachycardia can occur. Or when in case of regional uncoupling within the sinus node secondary pacemakers become active, mutual entrainment will cause sinus irregularity [19, 20]. Therefore, sinus tachycardias may be based on variations in intercellular coupling.

From the results of the present study it can be concluded that (1) the atrium electrotonically depresses the rate of diastolic depolarization of fibers in the border zone of the isolated rabbit sinus node; (2) within the intact isolated sinuse node the fibers in the border zone are capable of impulse generation with a higher frequency than fibers in the nodal center. This makes it desirable to use the adjectives 'latent' and 'dominant' pacemaker fibers only in the context of activation sequences.

References

1. Antzelevitch C, Jalife J, Moe GK (1982) Electrotonic modulation of pacemaker activity. Further biological and mathematical observations on the behavior of modulated parasystole. Circulation 66:1225–1232
2. Bleeker WK, Mackaay AJC, Masson-Pevet M, Bouman LN, Becker AE (1980) Functional and morphological organisation of the rabbit sinus node. Circ Res 46:11–22
3. Bonke FIM (1973) Electrotonic spread in the sinoatrial node of the rabbit heart. Pflügers Arch 339:17–23
4. Bonke FIM (1980) Electrophysiology of the sinus node and atrial pacemakers. In: Little RC (ed) Physiology of atrial pacemakers and conductive tissues. Futura Publishing Company, Mount Kisco NY, pp 171–186
5. Bonke FIM, Steinbeck G, Allessie MA, Mackaay AJC, Slenter VAJ (1982) The electrophysiological effects of cardiac glycosides on the isolated sinus node of the rabbit. In: Paes de Carvalho A, Hoffman BF, Lieberman M (eds) Normal and abnormal conduction in the heart. Futura Publishing Company, Mount Kisco NY, pp 347–361
6. Bouman LN, Gerlings ED, Biersteker PA, Bonke FIM (1968) Pacemaker shift in the sinoatrial node during vagal stimulation. Pflügers Arch 302:255–267
7. Bouman LN, Mackaay AJC, Bleeker WK, Becker AE (1978) Pacemaker shifts in the sinus node: Effects of vagal stimulation, temperature, and reduction of extracellular calcium. In: Bonke FIM (ed) The sinus node. Structure, function and clinical relevance. Martinus Nijhoff Medical Division, The Hague, pp 245–257

8. Bukauskas FF, Veteikis RP, Gutman AM, Mutskus KS (1977) Intracellular coupling in the sinus node of the rabbit heart. Biofizika 22:108–112
9. Capelle FJL van, Durrer D (1980) Computer simulation of arrhythmias in a network of coupled excitable elements. Circ Res 47:454–466
10. DeHaan RL, Fozzard HA (1975) Membrane response to current pulses in spheroidal aggregates of embryonic heart cells. J Gen Physiol 65:207–222
11. DeHaan RL, Hirakow R (1972) Synchronization of pulsation rates in isolated cardiac myocytes. Exp Cell Res 70:214–220
12. Deleze J (1970) The recovery of resting potential and input resistance in sheep heart injured by knife or laser. J Physiol 208:547–562
13. Delmar M, Jalife J, Michaels DC (1986) Effects of changes in excitability and intercellular coupling on synchronization in the rabbit sino-atrial node. J Physiol 370:127–150
14. De Mello WC (1972) The healing-over process in cardiac and other muscle fibers. In: De Mello WC (ed) Electrical phenomena in the heart. Academic Press, New York, pp 323–351
15. De Mello WC (1976) Influence of the sodium pump on intercellular communication in heart fibres: effect of intracellular injection of sodium ion on electrical coupling. J Physiol 263:171–197
16. De Mello WC (1983) The influence of pH on the healing-over of mammalian cardiac muscle. J Physiol 339:299–307
17. Griepp EB, Bernfield MR (1978) Acquisition of synchronous beating between embryonic heart cell aggregates and layers. Exp Cell Res 113:263–272
18. Irisawa A (1978) Fine structure of the small sinoatrial specimen used for the voltage clamp experiment. In: Bonke FIM (ed) The sinus node. Structure, function and clinical relevance. Martinus Nijhoff Medical Division, The Hague, pp 311–319
19. Jalife J (1984) Mutual entrainment and electrical coupling as mechanisms for synchronous firing of rabbit sino-atrial pacemaker cells. J Physiol 356:221–243
20. Jalife J, Michaels DC (1985) Phase-dependent interactions of cardiac pacemakers as mechanisms of control and synchronization in the heart. In: Zipes DP, Jalife J (eds) Cardiac electrophysiology and arrhythmias. Grune and Stratton, Orlando, pp 109–119
21. Jongsma HJ, Masson-Pevet M, Hollander CC, Bruyne de J (1975) Synchronization of the beating frequency of cultured rat heart cells. In: Lieberman M, Sano T (eds) Development and physiological correlates of cardiac muscle. Raven Press, New York, pp 185–196
22. Kodama I, Boyett MR (1985) Regional differences in the electrical activity of the rabbit sinus node. Pflügers Arch 404:214–226
23. Kreitner D (1985) Electrophysiological study of the two main pacemaker mechanisms in the rabbit sinus node. Cardiovasc Res 19:304–318
24. Mackaay AJC (1980) Frequency regulation in the sinus node. PhD Thesis University of Amsterdam
25. Masson-Pevet M, Bleeker WK, Mackaay AJC, Bouman LN (1979) Sinus node and atrium cells from the rabbit heart: a quantitative electron microscopic description after electrophysiological localization. J Mol Cell Cardiol 11:555–568
26. Noma A, Irisawa H (1976) Membrane currents in the rabbit sino-atrial node cell as studied by the double microelectrode method. Pflügers Arch 364:45–52
27. Sano T, Yamagishi S (1965) Spread of excitation from the sinus node. Circ Res 16:423–430
28. Sano T, Sawanobori T, Adaniya H (1978) Mechanism of rhythm determination among pacemaker cells of the mammalian sinus node. Am J Physiol 235:H379–H384
29. Seyama I (1976) Characteristics of the rectifying properties of the sino-atrial node cell of the rabbit. J Physiol (Lond) 255:379–397
30. Steinbeck G, Bonke FIM, Allessie MA, Lammers WJEP (1980) The effect of ouabain on the isolated sinus node preparation of the rabbit studied with microelectrodes. Circ Res 46:406–414
31. Strauss HC, Bigger JT (1972) Electrophysiological properties of the perinodal fibers. Circ Res 31:490–506
32. Takayanagi K, Jalife J (1986) Effects of digitalis intoxication on pacemaker rhythm and synchronization in rabbit sinus node. Am J Physiol 250:H567–H578
33. Toda N, Shimamoto K (1968) The influence of sympathetic stimulation on transmembrane potentials in the SA node. J Pharmacol Exp Ther 159:298–305
34. Weingart R (1977) The actions of ouabain on intercellular coupling and conduction velocity in mammalian ventricular muscle. J Physiol 264:341–365
35. Ypey DL, Clapham DE, De Haan RL (1979) Development of electrical coupling and action potential synchrony between paired aggregates of embryonic heart cells. J Membr Biol 51:75–96

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