

Functional and Morphological Organization of the Guinea-pig Sinoatrial Node Compared with the Rabbit Sinoatrial Node

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T. OPHOF, B. DE JONGE, A. J. C. MACKAAY, W. K. BLEEKER, M. MASSON-PEVET, H. J. JONGSMA AND L. N. BOUMAN. Functional and Morphological Organization of the Guinea-pig Sinoatrial Node Compared with the Rabbit Sinoatrial Node. *Journal of Molecular and Cellular Cardiology* (1985) **17**, 549–564. The primary pacemaker, i.e. the group of pacemaker cells discharging the sinoatrial node comprises less than 1000 cells in the guinea-pig and about 5000 cells in the rabbit. These primary pacemaker cells are described as 'central nodal' cells in light microscopy and as 'typical nodal' cells in electron microscopy. The action potential of the leading cells has a higher upstroke velocity in the guinea-pig than in the rabbit (6.2 v. 1.9 V/s). Gap junctions have been observed even in the very center of the node in both species. A zone of double-component action potentials at the septal margin of the node was observed in the rabbit, but not in the guinea-pig. Evidence is presented for abrupt transitions in electrophysiological as well as in ultrastructural characteristics in the guinea-pig sinoatrial node. The differences in intrinsic cycle length between both species but also between individuals of the same species are discussed.

KEY WORDS: Sinoatrial node; Primary pacemaker; Sinoatrial conduction.

Introduction

Among mammalian species there is a large variability with respect to shape, magnitude, vascularization and innervation of the sinoatrial node [4, 8]. Correlative studies taking into account electrophysiological, light microscopic and electron microscopic data are confined to the rabbit [2, 26]. From the literature it seems that there are species-dependent differences concerning the structure of sinoatrial nodes. James *et al.* [9] have described the canine and human sinoatrial node as a structure consisting of clusters of cells of different types, which may directly neighbor each other. The rabbit sinoatrial node also contains different cell types [18, 19], but the transition from one cell type into another is gradual. It is unknown whether these structural species differences have consequences for sinoatrial node function in different species. Information

about the relation between structure and function is lacking in other species than the rabbit [2, 26]. We chose the guinea-pig for this correlative study because a superfusion technique as used by us in the rabbit [2] is expected to be suitable also in a thinner sinoatrial node. We wanted to investigate whether the guinea-pig sinoatrial node is merely a scaled-down copy of the rabbit sinoatrial node with respect to both structure and function, or that the population of cell types is ordered in such a manner that a different structure and/or function results. Like the rabbit the guinea-pig has a sinoatrial node occupying all the space between epicardium and endocardium [7]. Since the activation pattern of the rabbit sinoatrial node can be described in two dimensions [2], the same is expected for the guinea-pig sinoatrial node.

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Methods

Nomenclature

In the literature many different names are used to describe different morphological cell types or cells with different electrophysiological characteristics. Since it is clear that the electrophysiological, light microscopic and ultrastructural models of the sinoatrial node are not completely superimposable [2] we consider it necessary to use terms exclusively related to the applied technique.

At the electrophysiological level we will distinguish the *primary pacemaker* as the group of earliest-discharging cells with identical action potential configuration. All other cells with an action potential configuration different from the primary pacemaker cells, but still showing diastolic depolarization will be referred to as *latent pacemakers*.

When we describe the light microscopic aspect of the sinoatrial node we will use the term *nodal cells* (i) where the cells are pale; (ii) where many nuclei close to each other are present and (iii) where many collagen fibers are present. If these nodal cells are in close interwoven apposition we will call them *central nodal cells*. When they are arranged in parallel, we will call them *peripheral nodal cells*.

At the ultrastructural level we will discriminate between typical nodal cells and transitional cells. *Typical nodal cells* are characterized by their small size and the lowest density of myofilaments showing no ordered organization. Cells intermediate in myofilament density, organization and orientation [19] between typical nodal cells and atrial myocardial cells will be referred to as *transitional cells*.

Preparation

In this study we used 40 guinea-pigs (540 ± 120 (S.D.) g) and 25 rabbits (2.5 to 3.5 kg). The guinea-pigs were decapitated. The heart was quickly taken out and superfused with a balanced salt solution. The rabbits were anesthetized with Hypnorm[®] (10 mg of fluanison + 0.2 mg of fentanyl base i.m./kg body w). Under artificial ventilation the chest was opened and the heart was quickly taken out. The preparations included the intercaval region but not the atrioventricular node and

only a small part of the interatrial septum. It was mounted on a perforated silicon rubber block in a tissue bath, endocardial side up. The upper part of the crista terminalis was cut to open the superior vena cava in order to obtain a good exposure of the sinotrial node. The bathing solution was modified after McEwen [21] and had the following millimolar composition: NaCl 130.6; NaHCO₃ 24.2; KCl 5.6; CaCl₂ 2.2; MgCl₂ 0.6; glucose 11.1; sucrose 13.2. It was saturated with a mixture of 95% O₂ and 5% CO₂. The pH was 7.4, the perfusion rate was 1200 ml/h and the experimental temperature was kept at 38 ± 0.1 °C.

Recording of electrical activity and data processing

Recording of electrical activity and data processing were performed as described previously [2]. All recorded electrical signals were stored on magnetic tape (Ampex FR 1300, frequency response 0 to 5000 Hz) for off-line computer analysis (sample frequency 2000 Hz). The action potential duration (APD) was measured (i) from the moment of V_{hfamp}, i.e. the potential halfway the maximum diastolic potential (MDP) and the top of the action potential, during the upstroke till V_{hfamp} during the repolarization phase of the action potential (APD 50) and (ii) from V_{hfamp} during the upstroke till the moment of the maximum diastolic potential (APD 100). The rate of diastolic depolarization (DDR) was measured during the first 100 ms after MDP in the guinea-pig and during the first 200 ms after MDP in the rabbit. Results are presented as means \pm one standard error of the mean, unless otherwise stated.

Mapping of the propagation of excitation

For the construction of a map of the propagation of excitation we used the recordings of the transmembrane potential of as many cells as could be impaled within the duration of the experiment. The activation moment (V_{hfamp}; [2]) was calculated by computer analysis. The values obtained were plotted on a grid according to the coordinates of the impaled site. In the guinea-pig we explored an area of about 3×4 to 5 mm in 13 preparations. In

the rabbit we explored an area of about 4×6 mm in 11 preparations. We made 80 to 140 impalements 0.4 mm (guinea-pig) and 0.8 mm (rabbit) apart from each other at the outer margins of this grid. In the primary pacemaker region, where propagation is slow, mapping was more refined. The distance between two impalements here was 0.05 or 0.1 mm. The lateral movements of the micro-manipulator were accurate to 0.01 mm. Six guinea-pig preparations were used for light microscopic and three for electron microscopic examination. Light and electron microscopic data of the rabbit have been published previously [2, 18, 19].

Light microscopy

In order to correlate the histologic findings with the electrophysiological data, several points of the coordinate system of the activation map were marked on the preparations after the electrical recordings were obtained. We filled the microelectrode with a 1% solution of Alcian Blue in 0.5 M sodiumacetate buffer, pH 4.0 [13]. In order to lower the resistance of the microelectrode, its tip was cut off. We made 10 to 15 dots (diameter 20 to 50 μm) iontophoretically on the endocardial surface by applying rectangular current pulses during 5 to 10 s with a frequency of 500 Hz and an impulse duration of 30 to 300 μs . The impulse strength was 0.5 mA. After completion of the electrophysiological part of the experiment, the preparations were fixed in buffered formalin. After removing the rubber block, the preparations were dehydrated and embedded in paraffin wax and serially sectioned. In the guinea-pig the sections (7 μm) were perpendicular to the crista terminalis (five experiments) and parallel to the crista terminalis (one experiment). In the rabbit the sections (10 μm) were perpendicular to the crista terminalis, (seven experiments). Every tenth section was stained for elastin tissue and counterstained according to Van Gieson [12]. After location of the blue dots in the sections, it was possible to determine the site of the section and the actual direction of sectioning with respect to the coordinate system; furthermore, the amount of shrinkage of the preparation caused by fixation and embedding could be approximated. We were able to

project the light microscopic structure onto the grid of the activation map. A two-dimensional reconstruction of the morphology of the guinea-pig sinoatrial node was made.

Electron microscopy

In three guinea-pig preparations several sites were marked with Alcian Blue (see above) after completion of the electrophysiological mapping.

These preparations were immersed in cold 2% paraformaldehyde and 2.5% glutaraldehyde in 0.15 M cacodylate buffer, pH 7.4 [10], during one night. Afterward the preparations were washed in 0.15 M cacodylate buffer, pH 7.4, and at the selected sites strips of 2 to 3 mm length and 0.4 mm width, with the long axis perpendicular to the crista terminalis, were cut out. These strips contained the complete tissue from endocardium to epicardium. Along the long axis of the strips blue dots were present at distances of 0.2 mm. These strips were then post-fixed in 1% OsO₄, for 1 h at 4°C, washed again in 0.15 M cacodylate buffer, pH 7.4, immersed in 0.5% magnesium uranyl acetate in 0.9% NaCl at room temperature during 20 min, and embedded in Araldite [7]. The sections were stained with lead citrate [24].

Quantification

The percentage of cell volume occupied by myofilaments was calculated using the technique of point counting with a square lattice [27]. A 1 × 1 cm lattice was used. For each site quantified, 20 electron micrographs of 18 × 18 cm were used at a final magnification of × 24 750. Magnification was calibrated using a germanium shadowed carbon replica (54864 lines/inch).

Results

Electrophysiology

Intrinsic frequency. In 40 guinea-pig preparations we measured a cycle length of 275 ± 41.8 (s.d.) ms at the moment of impalement of the primary pacemaker. In the rabbit this figure was 386 ± 31.0 (s.d.) ms. The cycle length in the guinea-pig right atrium preparation was constant, whereas the cycle length

of the rabbit right atrium preparation increased 10 to 15 ms/h. The preparations showed a regular beat-to-beat rhythm. The coefficient of variation of the mean cycle length, measured over 500 consecutive intervals, amounted to less than 0.1% in the guinea-pig and less than 0.4% in the rabbit.

The primary pacemaker. Cells that reached V_{hfamp} earliest (★, Fig. 1) covered an area, of only 0.02 mm^2 in the guinea-pig and of 0.1 mm^2 in the rabbit. We previously estimated that the amount of cells in the primary pacemaker area of the rabbit was about 5000. We estimate that this figure is less than 1000 in the guinea-pig because the primary pacemaker area is five times smaller and because the sinoatrial node is thinner in the guinea-pig. Table 1 shows the characteristics of the primary pacemaker in both species. Apart from the action potential amplitude all parameters were significantly different (Student's *t*-test, lowest significance level 0.05). The difference in intrinsic cycle length between the rabbit and the guinea-pig seems therefore based upon a less negative maximum diastolic potential, a shorter action potential duration, but also a higher rate of diastolic depolarization in the guinea-pig. There was a significant correlation between cycle length and APD 50 (APD 50 = 0.10 Cycle length + 55.3; $r = 0.546$; $P < 0.0005$; $n = 39$), between cycle length and APD 100 (APD 100 = 0.24 Cycle length + 70.7; $r = 0.758$; $P < 0.0005$; $n = 40$) and between cycle length and DDR (DDR = -0.84 Cycle length + 351.4; $r = -0.770$; $P < 0.0005$; $n = 40$) in the guinea-pig only. Cycle length and the maximum diastolic potential of the primary pacemaker cells of the separate guinea-pig preparations turned out to be independent of each other. Differences in intrinsic cycle length between different guinea-pig sinoatrial node preparations seem therefore mainly due to differences in the rate of diastolic depolarization and duration of the action potential.

Highest rate of diastolic depolarization and lowest rate of upstroke velocity. Figure 2 shows DDR-(left) and \dot{V}_{\max} -values (right) in the guinea-pig represented by oblique bars. Comparison with the activation map (Fig. 1) shows that the area of the primary pacemaker cells lies

within the zone of high DDR- and low \dot{V}_{\max} -values. In seven experiments we measured the total area in which the fibers had a \dot{V}_{\max} value lower than 10 V/s . It amounted to 1.0 ± 0.09 (s.e.) mm^2 .

This area was about 6 mm^2 in the rabbit (deduced from [2]). In the rabbit the primary pacemaker is also located within the zone of high DDR- and low- \dot{V}_{\max} -values [2].

The activation pattern. In all guinea-pig experiments the spread of excitation followed the same pattern (Fig. 1). The site of earliest discharge was located 0.4 to 1.2 mm away from the crista terminalis. From this site the impulse was preferentially conducted transversely to the crista terminalis. Next it propagated very fast along the crista terminalis in the superior as well as into the inferior direction. The average distance of propagation 10 ms after the discharge of the primary pacemaker was 1.6 mm. The septal side of the preparation was activated via a circuitous pathway around the inferior part of the sino-atrial node only. The tissue at the superior end of the node is the last to be activated. This preferential caudo-cranial propagation of the impulse at the septal border of the node is supported by Fig. 3. It is shown that the interruption of the pathway, made by an incision more than 1 mm caudal to the primary pacemaker and parallel to the crista terminalis, increases the conduction time between the primary pacemaker and the sites in the grey area by 10 to 15 ms. This is not to be expected if the impulse travelled along the shortest pathway between those sites. We did observe action potentials with a fast and slow component in the upstroke (see Fig. 1, action potential marked (c)) like those described by Lipsius and Vassalle [14]. These action potentials could be recorded only at the crista terminalis side of the node.

In the rabbit heart the spread of excitation followed a pattern that was essentially different from that observed in the guinea-pig (Figs. 1 and 4). In all preparations the impulse propagated preferentially from the site of earliest discharge that was located 0.5 to 2.0 mm away from the crista terminalis, into an oblique cranial (superior) direction towards the crista terminalis. The propagation of the impulse was blocked towards the

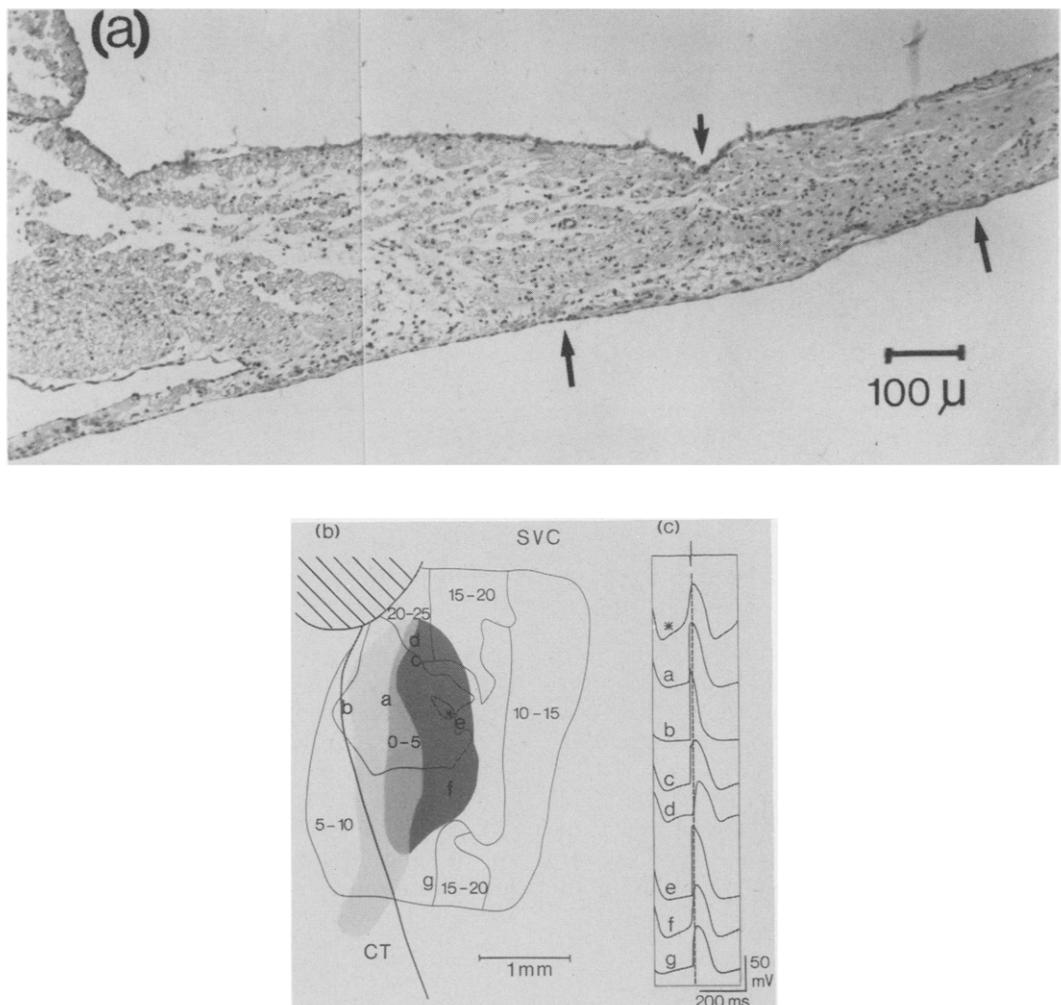


FIGURE 1. (a) Light micrograph of a section through the primary pacemaker of a guinea-pig right atrium cut perpendicular to the crista terminalis. The small arrow indicates the site of the Alcian Blue dot imposed iontophoretically at the position of the primary pacemaker, located by microelectrode recording. The crista terminalis is to the left and the interatrial septum to the right. The region between the two larger arrows is filled with central nodal cells from endocardium (top) to epicardium (bottom). (b) Activation map of a guinea-pig sinoatrial node. The asterisk indicates the location at which the impulse originates. The numbers (0-5, 5-10, 10-15, 15-20 and 20-25) in the areas between the isochrones indicate the interval in ms between the time of depolarization at \star and the time of depolarization in that area. The designation '5 to 10' means, for instance, that all cells in this area activate more than 5, but less than 10 ms after the primary pacemaker. The area of primary pacemaker cells (i.e. with characteristic spontaneous depolarization and first to depolarize, see the trace labelled \star in (Fig. 1(c)) is approximately 0.02 mm^2 . There is preferential conduction towards the crista terminalis and towards the inferior margin of the node (compare with Fig. 3). The shading represents a superimposition of the morphological evidence on the electrophysiological map, the darkest shading representing the central nodal cells, and the lightest shading representing the peripheral nodal cells. The intermediate shading delineates the region of overlap where central nodal cells are found on the epicardial side and peripheral cells on the endocardial side. CT: crista terminalis; SVC: superior vena cava. (c) Action potentials recorded at the sites indicated [\star and (a to g)] on Figure 1(b). The uppermost trace is the atrial electrogram. The vertical dashed line represents a time reference corresponding to the time of steepest rise of the atrial electrogram. Towards the crista terminalis there is an increase in upstroke velocity and a decrease in diastolic depolarization rate (compare traces (a) and (b), but also Fig. 2). Small, slowly rising action potentials are typical of the activity seen at the superior margin of the node (trace (d)). These cells always activate after the right atrium. It can be noticed that the impulse originates from central nodal cells. However, cells with diastolic depolarization and with relatively long action potential durations can be observed outside the light-microscopically defined nodal area (trace (g)). On the other hand, cells with slow diastolic depolarization and with high upstroke velocity can be observed very close (about 0.1 mm) to the primary pacemaker (trace (e)).

TABLE 1. Characteristics of the primary pacemaker in the guinea-pig and the rabbit sinoatrial node under standard conditions

	Cycle Length (ms)	Action potential amplitude (mV)	\dot{V}_{\max} (V/s)	MDP (mV)	APD 50 (ms)	APD 100 (ms)	DDR (mV/s)
	SACT (ms)	(ms)					
Guinea-pig	275 ± 6.6 (40)	12 ± 0.8 (40)	67 ± 1.6 (40)	6.2 ± 0.54 (40)	-57 ± 1.3 (33)	82 ± 1.2 (39)	137 ± 2.1 (40)
	★	★		★	★	★	★
Rabbit	386 ± 6.2 (25)	32 ± 1.8 (25)	66 ± 1.9 (25)	1.9 ± 0.19 (25)	-61 ± 1.4 (11)	105 ± 3.7 (14)	180 ± 6.3 (14)

The number of observations is between parentheses. Mean values are given ± one s.e.

SACT: sinoatrial conduction time;

\dot{V}_{\max} : maximal upstroke velocity of the action potential;

MDP: maximum diastolic potential;

APD: action potential duration;

DDR: diastolic depolarization rate.

*: significant difference between the two species (lowest significance level 0.05).

interatrial septum. In the blocking zone double-component action potentials were recorded. On average the impulse had travelled 0.7 mm 10 ms after the discharge of the primary pacemaker.

Towards the septal border of the node action potentials quite different from the primary pacemaker action potentials were recorded at only 0.05 mm septal from the

primary pacemaker in the guinea-pig [Fig. 5(a)]. In the rabbit these differences were not observed even at a distance of 0.4 mm septal from the primary pacemaker [Fig. 5(b)].

Light microscopy

At the light microscopic level the central part of the guinea-pig node was recognized as a

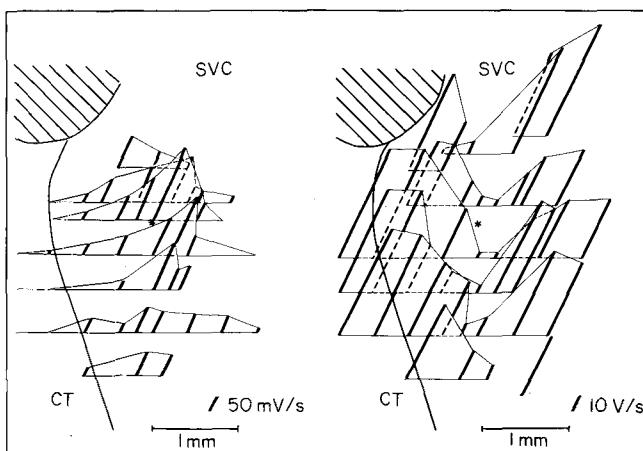


FIGURE 2. Spatial distribution of DDR (left) and \dot{V}_{\max} (right) within the guinea-pig sinoatrial node. The oblique bars indicate the values of DDR (mV/s) and \dot{V}_{\max} (V/s). The height of the oblique bars (foot to top) is proportional to the value of DDR and \dot{V}_{\max} . The plot is made on the grid of Figure 1, which describes the same preparation. CT: crista terminalis; SVC: superior vena cava. The asterisk indicates the site of the primary pacemaker. Calibration bars are at the bottom of the figure.

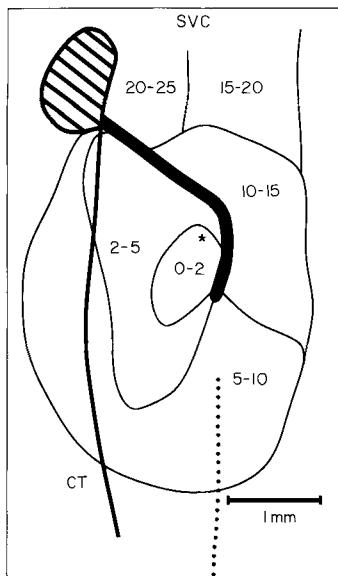


FIGURE 3. Illustration of the preferential caudo-cranial (inferior-superior) direction of propagation at the septal border of the guinea-pig sinoatrial node. The activation times (0-2, 2-5, 5-10, 10-15, 15-20 and 20-25 ms) are given in the areas between the isochrones. The numbers indicate the spread of excitation in ms from the primary pacemaker, marked \star , towards the crista terminalis and the other parts of the nodal region under normal conditions. The designation '2 to 5' means, for instance, that all cells in this area activate more than 2, but less than 5 ms after the primary pacemaker. The total nodal region is activated within 25 ms in this preparation. An incision (black dots) made caudal to the primary pacemaker causes an increase in activation times by approximately 15 ms in the area above the thick line. This means that all cells that activated 15 to 20 ms after the primary pacemaker before the cut, activated about 30 to 35 ms after the primary pacemaker when the cut had been made. CT: crista terminalis; SVC: superior vena cava.

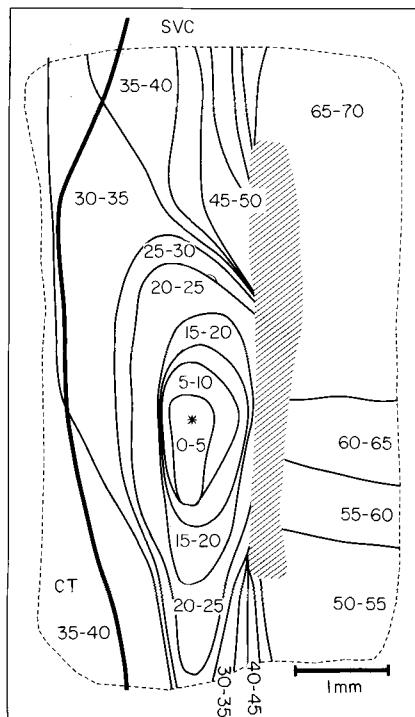


FIGURE 4. Activation pattern of a rabbit sinoatrial node. The impulse originated from the site marked \star . The activation times (0-5, 5-10, 10-15 and so on) are given in the areas between the isochrones. For instance, the designation '20 to 25' means that all cells in this area activate more than 20, but less than 25 ms after the primary pacemaker. The hatched area refers to a zone where action potentials with double components during the repolarization phase are recorded [2, 3]. Not all areas are numbered for reason of clarity. The thick line is the crista terminalis (CT). SVC: superior vena cava. Note the detour of conduction around the superior and inferior border of the hatched area [3].

structure of interweaving cells embedded in a network of collagen fibers. Towards the crista terminalis a zone of more parallel arranged cells could be detected (peripheral nodal cells) (Fig. 1). There is a small zone (0.1 to 0.4 mm, see Fig. 1) where the central nodal cells at the epicardial side cover the peripheral nodal cells. In this aspect the guinea-pig clearly differs from the rabbit. At the septal side we were not able to detect peripheral nodal cells.

In contrast to Anderson [1] we did not observe a typical sinoatrial artery, either a central one, or one running among peripheral nodal cells.

In the rabbit we always observed a zone of peripheral nodal cells at all sides of the central nodal cells. This zone was broader at the crista terminalis side than at the septal side. Further we never observed overlapping layers of central and peripheral nodal cells. Comparative data on the light microscopic structure are given in Table 2.

The primary pacemaker lies within the zone of central nodal cells in both species (Fig. 1 and [2]). Comparison of Figures 1 and 2 and data from [2] show that also the areas of low V_{max} and high rate of diastolic depolarization are within the zone of central nodal cells in both species.

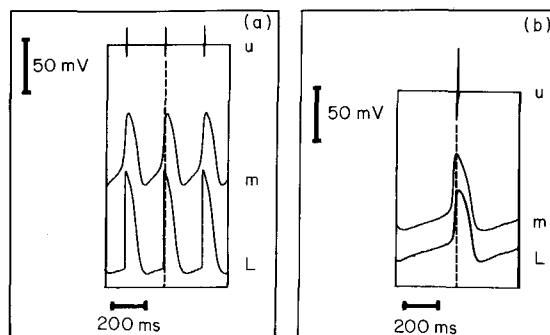


FIGURE 5. The transition in action potential configuration from the primary pacemaker towards the interatrial septum. (a) guinea-pig; (b) rabbit; Upper trace(u): atrial electrogram; Middle trace(m): action potential from the primary pacemaker; Lower trace(L): action potential from a cell located 0.05 mm (guinea-pig) or 0.40 mm (rabbit) from the primary pacemaker, on the septal side. The action potentials (the second in (a)) were superimposed on the moment of the corresponding atrial discharge. The atrial electrograms were made during the impalement of the primary pacemakers.

Electron microscopy

Figure 6 shows an example of the ultrastructure of the typical nodal cells from the site of the primary pacemaker in the guinea-pig. The neighboring cardiac cells were of one type only. The structure of the guinea-pig sinoatrial node is thus comparable with the structure of the rabbit sinoatrial node [18, 19]. The cells were essentially the same as in the rabbit, i.e. they had a long axis of 20 to 30 μm , an irregular profile in cross-section and a diameter smaller than 8 μm). Small gap junctions were present between the typical nodal cells in the primary pacemaker area.

The most prominent feature of primary pacemaker cells is their emptiness [19]. In primary pacemaker cells from the rabbit more than 50% of the cell volume is free of organized structures and it was shown that

this 'emptiness' was especially the consequence of the low amount of myofilaments [19]. In three guinea-pig preparations we also quantified the percentage of myofilaments at many sites. We counted 28 ± 1.5 (S.E.) % of myofilaments in the primary pacemaker center. In the rabbit 27% of myofilaments have been observed in the primary pacemaker [19], but there were other sites in the preparations where the percentages of myofilaments were 20 to 25% [19]. Like in the rabbit [20] we observed many caveolar invaginations in the guinea-pig. Poor organization and orientation of the myofilaments was observed in both species. Figure 7 shows the percentage of myofilaments as quantified in 35 sites in one of the three guinea-pig preparations with superimposed the activation pattern of this preparation. The primary pacemaker was found within the area of lowest myofilament density. The areas of typical nodal cells with less than 25% myofilaments cover only about 0.2 mm^2 in the guinea-pig (deduced from Fig. 7) and about 2.5 mm^2 in the rabbit [19].

Control experiments without prior electrophysiological study have shown that the typical characteristics of cells in the rabbit primary pacemaker area are no artefacts due to long experimentation [2]. However, long experimentation does widen the extracellular space. Such control experiments are difficult to perform in the guinea-pig, because the area of 'empty cells' is so small that one cannot estimate its location by eye, which is necessary in a control experiment without electrophysiological data.

At a distance of only 0.2 mm from the primary pacemaker center to the crista terminalis different cells could be observed at the epicardial and endocardial side of the prep-

TABLE 2. Area of the sinoatrial node by light microscopic criteria.

	Area of the central nodal cells (mm^2)	Area of the total node (mm^2)	Maximal length (mm)	Maximal width (mm)	Thickness* (mm)
Guinea-pig ($n = 6$)	2.1 ± 0.26	3.5 ± 0.31	4	1	0.10–0.22
Rabbit ($n = 7$)	3.5 ± 0.40	circa 10	circa 6	2	0.10–0.30

Data are given as mean \pm one S.E.

* The thickness of the tissue was measured at the site of the primary pacemaker center. In all directions the thickness of the tissue increases.

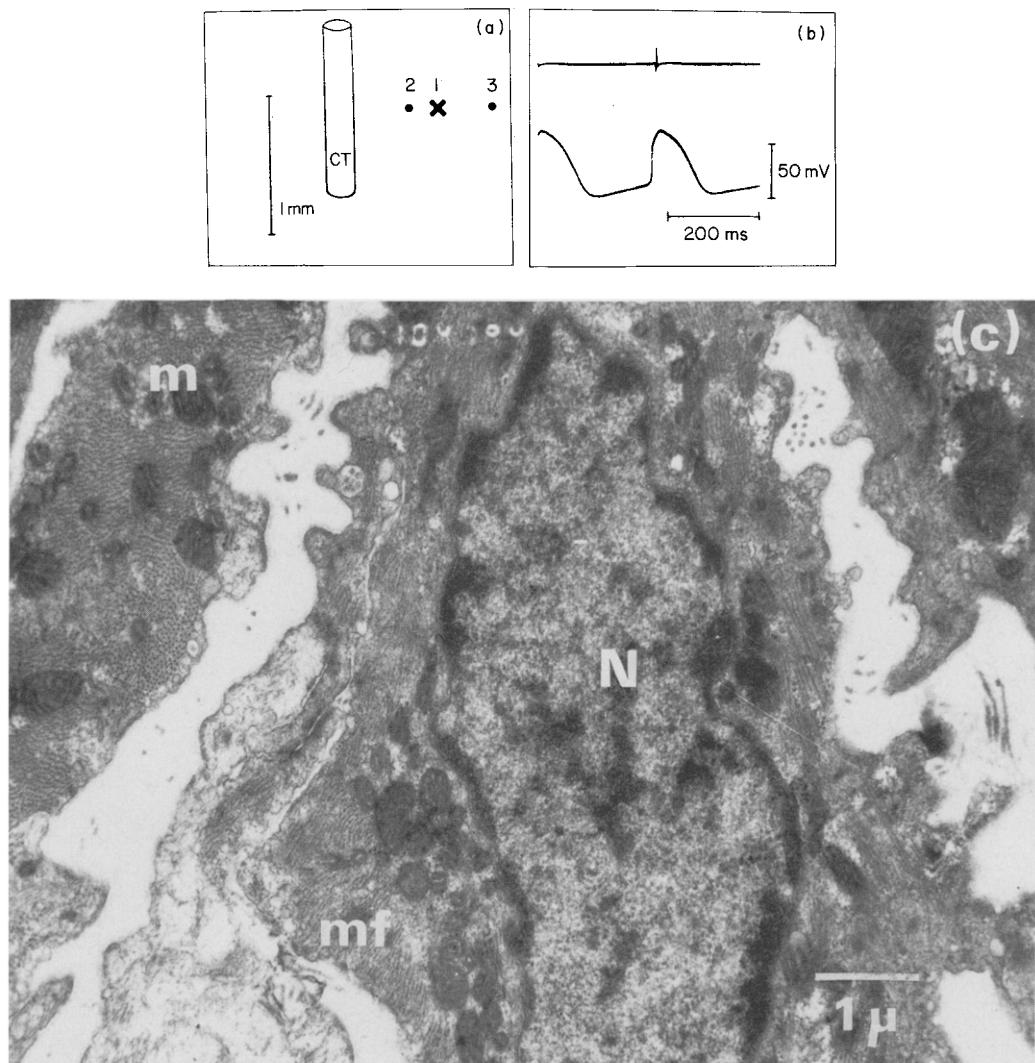


FIGURE 6. (a) Diagram (guinea-pig) pertinent to Figures 6, 8 and 9. In this figure site 1 (\times) is described. Site 2 is described in Figure 8 and site 3 in Figure 9. Site 1 (\times) is the site where the primary pacemaker was located. Action potentials were recorded (b) and electron micrographs were made (c). CT: crista terminalis. (b) Action potential and atrial electrogram. This primary pacemaker cell is an example of a leading cell with a rather high V_{max} of 9.8 V/s (see also Table 1). Such large upstroke velocities are never observed in the rabbit primary pacemaker. (c) Electron micrograph showing parts of at least three adjacent cells. mf: myofilaments; m: mitochondria; N: nucleus.

aration. Cells at the endocardial side contained 43% myofilaments, whereas this figure was 22% at the epicardial side [Fig. 8, (d) and (e)]. The action potentials at the endocardial side [panel (b)] had a markedly higher V_{max} value than the action potentials at the epicardial side [panel (c)]. The impalements were made from the endocardial side of the preparation. We first recorded the action potential in [panel (b)] and then, by moving

the microelectrode further inside the tissue, the action potential in [panel (c)]. At this same site we counted in 20 electron micrographs three times as many cell parts per surface area at the epicardial side than at the endocardial side, which means that the epicardially located cells are much smaller and/or far more interdigitated. At the crista terminalis border of the primary center we found zones consisting exclusively of collagen

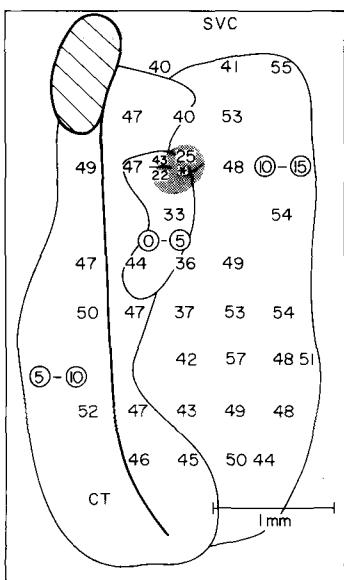


FIGURE 7. The percentage of myofilaments, i.e. the percentage of intracellular volume occupied by myofilaments, at 35 sites in the same guinea-pig preparation as used in Figures 6, 8, 9, 10 and 11. The grey area is the area of the typical nodal cells. This area covers about 0.2 mm². The activation pattern is superimposed. The impulse originates in the typical nodal cells at the site marked with the asterisk. The activation times (0–5, 5–10 and 10–15 ms) are given in the areas between isochrones. For instance, the designation '5 to 10' means that all cells in this area activate more than 5, but less than 10 ms after the primary pacemaker. CT: crista terminalis. SVC: superior vena cava.

fibers between epicardially located typical nodal cells and endocardially located transitional cells, but there were also sites where we observed a gradual transition in cell type from endocardium to epicardium. This suggests that the contact zone between typical nodal cells and transitional cells does not extend over the whole length of the crista terminalis border of the node. Figure 9 shows an electron micrograph 0.4 mm towards the septum from the primary center. It is an atrial cell as it lacks the characteristic of diastolic depolarization and it contains 48% of myofilaments, which are all organized in myofibrils and orientated in the superior-inferior direction of the preparation. Many sarcomeres can be observed. Typical of a rather abrupt transition in cell type is the electron micrograph in Figure 10, showing an empty cell bordering a cell well-filled with myofibrils. Between those cells collagen fibers and the glycocalyx can be

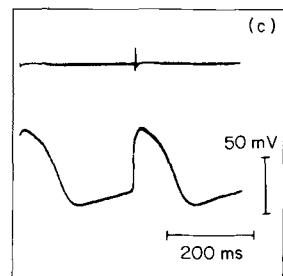
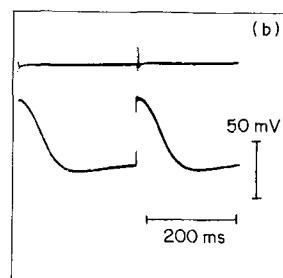
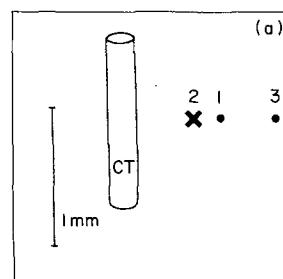
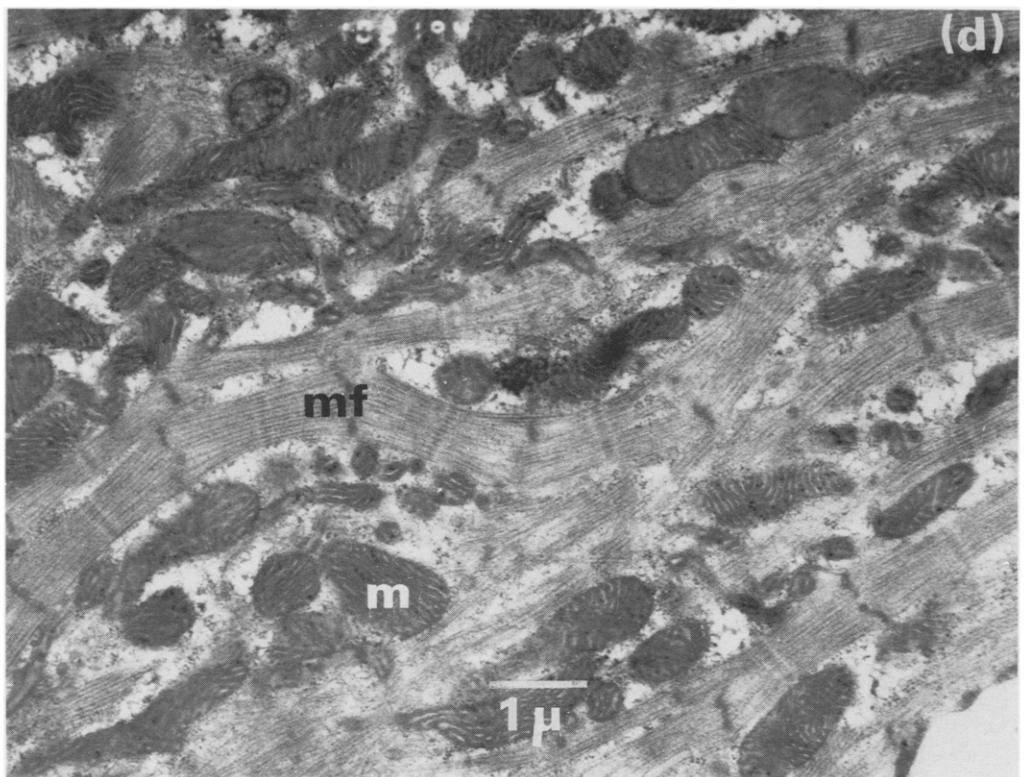
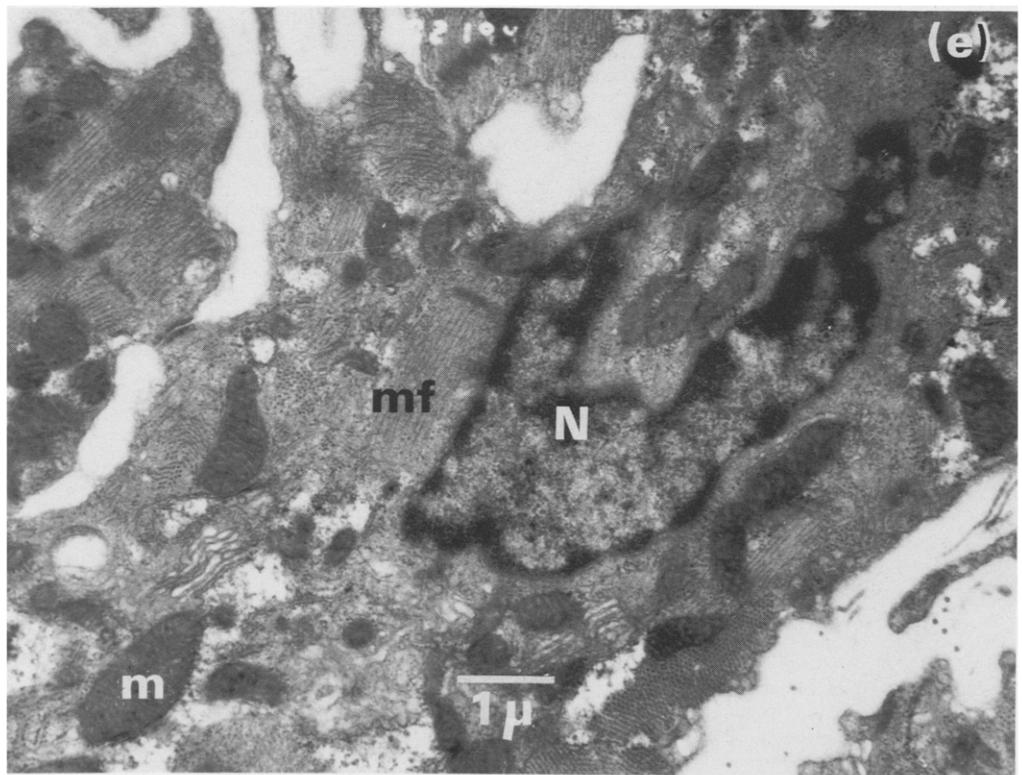


FIGURE 8. (a) Diagram (guinea-pig) pertinent to Figures 6, 8 and 9. In this figure site 2 (X) is described. Site 1 was described in Figure 6 and site 3 is described in Figure 9. Site 2 (X) is located 0.2 mm from the primary pacemaker (which was located at site 1), on the crista terminalis side. Action potentials were recorded (b) and (c) and electron micrographs were made (d) and (e). CT: crista terminalis. (b) Action potential and atrial electrogram. The action potential was recorded from the endocardial side of the preparation. (c) Action potential and atrial electrogram. The action potential was recorded at the epicardial side of the cell from which the action potential in (b) was recorded. To this end we moved the microelectrode deeper into the preparation during the impalement of the cell described in (b). (d) Electron micrograph showing a transitional cell (all myofilaments are organized in myofibrils and are running parallel to one another). This electron micrograph was made from cells close to the endocardium. mf: myofilaments; m: mitochondria. (e) Electron micrograph showing a typical nodal cell (myofilaments in all directions, empty cell). This electron micrograph was made from cells close to the epicardium. mf: myofilaments; m: mitochondria; N: nucleus.

(d)



(e)



observed. Such abrupt transitions are not present in the rabbit.

The rate of diastolic depolarization is related to the percentage of myofilaments (Fig. 11). For comparison Figure 11 gives also the relation between the percentage of myofilaments and the rate of diastolic depolarization as reported in the rabbit [19].

Ultrastructural data concerning the rabbit were reported previously [2, 18-20].

Discussion

Electrophysiology

Method. Our method of mapping the electrical activity by means of multiple impalements with one microelectrode is only valid when every excitation has the same origin and path of propagation. In 40 guinea-pig and 25 rabbit preparations the location of the primary pacemaker remained stable during

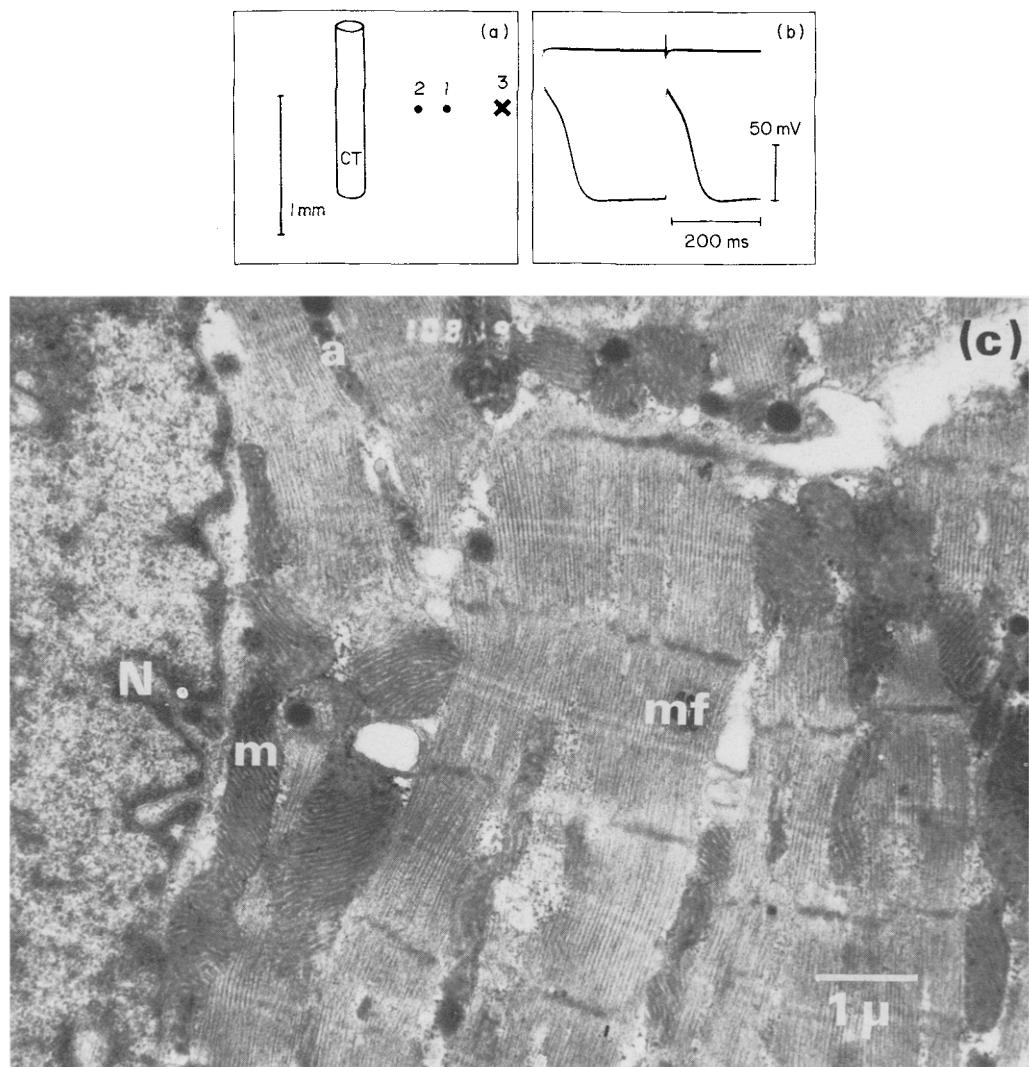


FIGURE 9. (a) Diagram (guinea-pig) pertinent to Figures 6, 8 and 9. In this figure site 3 (x) is described. Site 1 was described in Figure 6 and site 2 in Figure 8. Site 3 (x) is located 0.4 mm from the primary pacemaker (which was located at site 1), on the septal side. Action potentials were recorded (panel b) and electron micrographs were made (c). CT: crista terminalis. (b) Action potential and atrial electrogram. There is little phase-4 depolarization. (c) Electron micrograph showing an atrial cell (many myofibrils in the same direction, atrial granulae). a: atrial granula; mf: myofibrils; m: mitochondria; N: nucleus.

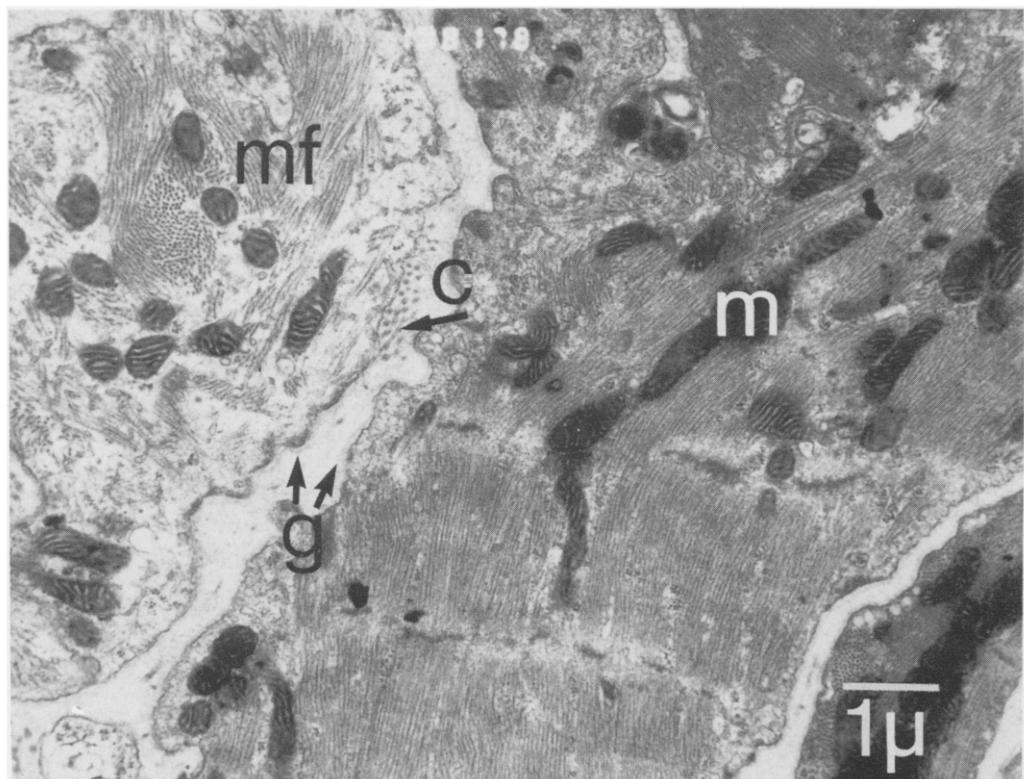


FIGURE 10. Example of abrupt transition in cell type in two adjacent cells in the guinea-pig. Typical nodal cell (left) and transitional cell (right). Note collagen fibers [(c) and long arrow] and glycocalyx [(g) and short arrows] between the cells. mf: myofilaments; m: mitochondria.

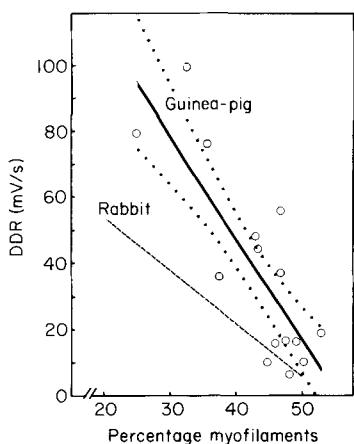


FIGURE 11. Relation between the rate of diastolic depolarization (DDR) and the percentage of intracellular volume occupied by myofilaments. Regression lines are given (solid: guinea-pig; dashed: rabbit). The guinea-pig regression line is based on those sites from Figure 7 with both ultrastructural and electrophysiological data. (Regression line $Y = -3.1 X + 173$; $r = -0.818$, $n = 15$). The circles indicate the data points. The dotted lines indicate the value of one s.e.m. The rabbit data were taken from [19]. (Regression line: $Y = -1.6 X + 86$, $r = -0.78$, $n = 40$).

the experiment as was confirmed by consecutive impalements during the experiment at the same site with time intervals up to several hours. Furthermore we observed that during continuous impalements the sinoatrial conduction time does not change more than 0.1 ms beat-to-beat, suggesting a stable pathway of conduction. The use of the membrane potential halfway the maximum diastolic potential and the top of the action potential as activation moment of a cell has been amply discussed previously [2].

The primary pacemaker. The primary pacemaker in the guinea-pig has a significantly higher \dot{V}_{\max} compared to the rabbit (6.2 v. 1.9 V/s; Table 1). Since the distance between two impalements in the very center of the node was only 0.05 mm and the area of the primary pacemaker cells covers about 0.02 mm^2 , it may be excluded that we failed to localize the primary pacemaker in the guinea-pig. The higher \dot{V}_{\max} -values in the guinea-pig primary pacemaker may be caused either by stronger

intrinsic membrane currents during fast depolarization or by weaker electrical coupling of these cells to the surrounding cells. From the fact that the impulse travels 1.6 mm in the guinea-pig v. 0.7 mm in the rabbit within 10 ms after the discharge of the primary pacemaker which does not indicate weaker electrical coupling in the guinea-pig primary pacemaker, we conclude that the kinetics and/or the number of channels of the depolarizing currents during \dot{V}_{\max} in the guinea-pig differ from those in the rabbit. Phase-4 depolarization from the moment of the maximum diastolic potential until the estimated threshold potential occurs at a more depolarized level in the guinea-pig compared to the rabbit (compare the magnitude of the maximum diastolic potential and the rate of diastolic depolarization in both species, Table 1). From this one would expect that less pacemaker current, whatever its nature, is available for the upstroke of the action potential in the guinea-pig primary pacemaker. This would be in agreement with lower \dot{V}_{\max} -values in the guinea-pig instead of the measured higher \dot{V}_{\max} -values.

When we compare the intrinsic cycle length in both species, we can compute that the difference in action potential duration (43 ms, Table 1) explains about 40% of the difference in cycle length (111 ms, Table 1) between the guinea-pig and the rabbit. The other 60% is explained by the less negative maximum diastolic potential and the higher diastolic depolarization rate in the guinea-pig, if it is assumed that there are no differences in threshold potential.

Regarding the primary pacemaker it is stressed that sinoatrial node function is studied under standard conditions. In the guinea-pig shifts of pacemaker dominance may be induced by the addition of adrenalin or acetylcholine or by changes in the ionic composition of the superfusion fluid, as is the case in the rabbit [15-17].

Electrophysiology and structure

Activation pattern and conduction of the impulse. We have described the activation pattern of the sinoatrial node in the guinea-pig [Figs 1 and 7] and in the rabbit (Fig. 4). In some experiments (like in Fig. 7) the complete guinea-pig intercaval region is activated within 15 ms, in others this takes 25 ms (Fig. 1). The impulse

originates from cells defined as central nodal cells by light microscopy (Fig. 1) and defined as typical nodal cells by electron microscopy (Fig. 7). Direct evidence for the correlation of the site of pacemaker dominance with the area of typical nodal cells has been provided in the rabbit previously by Taylor *et al.* [25].

The preferential pathway of the conduction of the impulse towards the crista terminalis in the guinea-pig (Fig. 12) cannot be explained by the existence of a zone of cells with low excitability at the septal border (Fig. 1). We were not able to detect peripheral nodal cells at the septal border of the guinea-pig node (Fig. 1) and the differences in percentages of myofilaments between equidistant sites were larger at the septal than at the crista terminalis border of the guinea-pig node (Fig. 7). Thus both from light and electronmicroscopic observations it appears that cells which are thought to be specialized in conducting the impulse towards the atrium are more abundant at the crista terminalis border than at the septal border of the guinea-pig node. Very different action potentials recorded over very small distances (0.05 mm, Fig. 5(a)) suggest low electrical coupling at the septal border of the guinea-pig sinoatrial node in combination with normal excitability (Figs 1 and 5). Experiments measuring the electrical coupling from the guinea-pig primary pacemaker

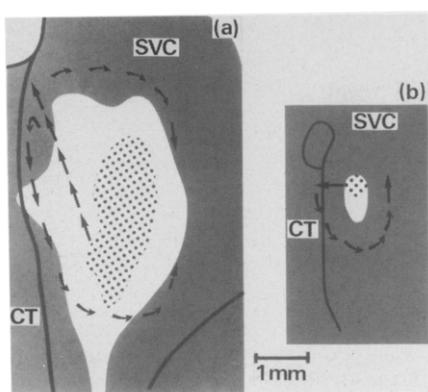


FIGURE 12. Comparison of the area of cells with less than 25% myofilaments (dots), 25% to 35% myofilaments (grey) and more than 35% myofilaments (dark grey) in the rabbit (a) and the guinea-pig (b). The percentages are with respect to intracellular volumes. The mm-scale at the bottom of the figure is the same for both species. The data were taken from [19] and from Figure 7 of this study. The schematic activation pattern (compare with Figs 1 and 4) is superimposed.

towards the crista terminalis and towards the interatrial septum, which coupling is equal in both directions in the rabbit [3], are needed to settle this point. The caudo-cranial (inferior-superior) direction of propagation at the septal border of the guinea-pig node (Figs 1, 3, 7 and 12) may be explained by little-excitatory cells at the cranial border of the guinea-pig node [Fig. 1, action potential (d)].

In the rabbit the impulse also originates from typical nodal cells [2, 18, 19, 25, 26]. At the septal side of the node, however, a zone of block is introduced by the existence of a zone of low excitability [3, 23]. The wave front encircles this zone of block both from the superior and inferior side (Figs 4 and 12). In the rabbit the transitions in action potential configuration are not abrupt as in the guinea-pig [Fig. 5(b)]. The same is valid for the transitions in morphology at the margins of the node. However, the structure of the rabbit and guinea-pig sinoatrial node in the central part is comparable. At any one site, within the central part, one does not observe typical nodal cells and working myocardial cells directly neighboring each other as has been reported in the canine and human sinoatrial node [9].

We observed that during the first 10 ms after the discharge of the primary pacemaker the impulse travels further in the guinea-pig (1.6 mm) than in the rabbit (0.7 mm). This seems to be caused at least in part by the higher V_{max} of the guinea-pig primary pacemaker (Table 1), which thus delivers a more adequate stimulus for neighboring cells.

Differences between the guinea-pig and rabbit sinoatrial node. The guinea-pig sinoatrial node differs from the rabbit sinoatrial node with respect to the following points: a faster rate of diastolic depolarization (mean 121 v. 83 mV/s, Table 1); a shorter action potential duration (137 v. 180 ms, Table 1); a shorter cycle length (mean 275 v. 386 ms, Table 1); a smaller zone of central nodal cells (2.1 v. 3.5 mm², Table 2); a smaller total area (3.5 v. about 10 mm², Table 2); absence of peripheral nodal cells at the septal border (Fig. 1); abrupt transitions in action potential configuration along the septal border (Fig. 5); different cell types at the endocardial and epicardial side occur in the guinea-pig at the

crista terminalis border of the node (Figs 1 and 8); absence of a zone of block with double-component action potentials; a very small area of typical nodal cells with less than 25% myofilaments (0.2 v. 2.5 mm², Fig. 7); activation of the septal border of the node via the inferior side only. The last two differences are summarized in Figure 12.

Difference in intrinsic cycle length. The reason why sinoatrial nodes of smaller animals have shorter cycle lengths than nodes of larger animals is unknown (Table 1). The action potentials of the rabbit and guinea-pig primary pacemakers differ in maximum diastolic potential, action potential duration and diastolic depolarization rate (Table 1, Fig. 11). It seems to us that the higher diastolic depolarization rate in the guinea-pig, although the maximum diastolic potential is less negative, points to at least quantitative differences in pacemaker currents between different species. It would be interesting to investigate whether there exists a special pacemaker current active during diastole in the guinea-pig, or to investigate whether there are important quantitative differences in the contribution of the three time-dependent pacemaker currents, i_f (inward current, activated by hyperpolarization negative to -50 mV), i_{si} (the slow inward current) and i_K (outward current) [5, 6] to the process of diastolic depolarization in the two species. Such quantitative differences might be pertinent to different species, but also to individuals within the same species and even to different intranodal sites within the same sinoatrial node. Intranodal differences in the relative contribution of the three time-dependent pacemaker currents seem beyond doubt since reports of Kreitner [11] and Brown *et al.* [6]. With respect to this we might have an explanation for the observed variability in intrinsic cycle length within the same species [22].

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