AGE-DEPENDENCE OF THE CHRONOTROPIC RESPONSE TO NORADRENALINE, ACETYLCHOLINE AND TRANSMURAL STIMULATION IN ISOLATED RABBIT ATRIA

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Abstract—The chronotropic response to noradrenaline, tyramine, acetylcholine and transmural electrical stimulation was compared in atria isolated from rabbits at different stages of development after birth (day 2 to day 210). Pacemaker rates under steady state conditions were related inversely to days after birth; the rate in atria from rabbits at day 2 was significantly greater than that at days 10-210. The rate of neonatal rabbit atria was not significantly reduced by propranolol and the positive chronotropic response to noradrenaline was not significantly different in atria from different ages of rabbits as far as threshold concentrations for inducing tachycardia and ED50's were concerned. The maximum rate induced by noradrenaline was higher in neonatal rabbit atria than in adult rabbit atria. The effect of tyramine was approx. the same regardless of age. Increase in the pacemaker rate induced by transmural neural stimulation varied directly with age. The negative chronotropic effect of acetylcholine was greater in neonatal than in adult rabbit atria; the ED50 in the former was significantly less than in the latter. Bradycardia induced by transmural stimulation of intracardiac cholinergic nerves was related directly to age. Tachycardia in the neonatal rabbit atria may be due to electrogenic characteristics of pacemaker cells which differ from those in adult rabbit atria. Our evidence strongly suggests that the adrenergic and cholinergic nerves innervating the S-A node develop at an early postnatal stage in the rabbit.

Findings obtained in different ages of mammals concerning the responsiveness of the heart to autonomic drugs are not always consistent in different species and under different experimental conditions. It has been demonstrated that the positive chronotropic effect of noradrenaline and isoproterenol is greater in neonatal dogs, anesthetized, adrenalectomized and vagotomized, than in adult dogs under the same procedures (1), whereas the heart in infant rats in situ is more sensitive to isoproterenol than in adult rats (2) but activation of adenylate cyclase by noradrenaline is less in broken cell preparations from young rats than with those from adult rats (3).

Autonomic innervation in the heart develops gradually after birth (4, 5), and the response to stimulation of the nerves in the heart in situ is altered with development (6, 7).

The present study describes comparisons of the pacemaker rate and the reactivity of sinoatrial (S-A) nodal pacemaker cells to noradrenaline, tyramine or acetylcholine as well as the stimulation of intracardiac adrenergic and cholinergic nerves in atria from rabbits at different stages of development.
MATERIALS AND METHODS

Albino rabbits of both sexes and different ages were sacrificed by a blow of the neck and exsanguinated from common carotid arteries. The entire heart was rapidly removed and atrial preparations were prepared. Ventricles were homogenized in ice-cold perchloric acid for the determination of noradrenaline. Atrial preparations were fixed between hooks in a muscle bath of 60 ml capacity containing the nutrient solution which was maintained at 30±0.5°C and aerated with a mixture of 95% O₂ and 5% CO₂. The resting tension was adjusted to 100-200 mg in atria at days 2-10, to 200-300 mg in atria at days 20-30 and to 300-400 mg at days 60 or over. Hooks anchoring the atrial appendage were connected to the lever of a force-displacement transducer (Nihonkoden Kogyo Co., Tokyo). The composition of the solution was as follows (mM): Na⁺, 162.1; K⁺, 5.4; Ca²⁺, 2.2; Cl⁻, 157.0; HCO₃⁻, 14.9; and dextrose, 5.6. The pH of the solution was 7.2 to 7.3. Before the measurements were taken, the preparations were allowed to equilibrate for 60 to 90 min in control media, during which time the solution was replaced every 15 to 20 min.

Intracardiac adrenergic and cholinergic nerves were transmurally stimulated by a bipolar silver electrode placed on the S-A node. Details of the procedure were described in an earlier report (8). A train of square pulses of supramaximum intensity with 0.1 msec duration, at frequencies of 5, 20 and 100/sec, was applied to the S-A node for 3 sec. Stimulus pulses were provided by an electrogenic stimulator (Nihonkoden Kogyo Co., MSE-3).

For the study with acetylcholine and transmural stimulation, electrical activities were recorded from the S-A node by the use of glass microelectrodes having resistances of 10 to 30 megohms. Atrial contractions and action potentials were displayed on an ink-writing oscillograph (Sanjo Sokki Co., Tokyo). Atrial rates were calculated by 10 measurements of the cycle length between contractions or action potentials. When the negative chronotropic response to transmural electrical stimulation was obtained, the maximum cycle length was measured and the atrial rate was calculated. Drugs were added directly to the bathing media in cumulative concentrations. Preparations had been treated for 20 min with cocaine, before the dose-response relationship of noradrenaline was obtained.

Ventricles were homogenized in 0.4 N perchloric acid for the assay of noradrenaline. Noradrenaline was extracted by the method of Anton and Sayre (9) with minor modification and assayed fluorometrically (10).

Results shown in the text, figures and table represent the mean values±standard errors of the means. Statistical analyses were made using the Student’s t test. Drugs used were dl-noradrenaline hydrochloride, tyramine hydrochloride, acetylcholine chloride, cocaine hydrochloride, dl-propranolol hydrochloride, metiamide and tetrodotoxin (Sankyo Co.).
RESULTS

Pacemaker rate in atria from rabbits of different ages

The rate of spontaneously beating atria from rabbits at different stages of development (day 2 to day 210) was related inversely to days after birth. As shown in Fig. 1, the rate of atria at day 2 (164±6.8 beats/min, N=14) was significantly different from the rate of 138±6.3 beats/min (N=8) at day 10 (P<0.05), 140±5.9 beats/min (N=13) at days 20-30 (P<0.02) 111±8.1 beats/min (N=9) at days 60-90 (P<0.001) and 103±3.8 beats/min (N=5) at days 180-210 (P<0.001). Tachycardia observed in neonatal rabbit atria was not influenced by treatment with 10^-6 M propranolol and 10^-5 M metiamide. Even when the resting tension was decreased to less than 50 mg, the rate was not significantly slowed.

Effects of noradrenaline, tyramine and transmural stimulation of adrenergic nerves

The addition of noradrenaline in concentrations ranging from 5×10^-9 to 10^-5 M caused a dose-related increase in the rate of atria at day 2: a significant increase was obtained at 5×10^-7 M and a maximum rate increase at 10^-5 M (Fig. 1). Similar results were obtained in atria from rabbits at other stages of development. The maximum rate induced by 10^-5 M noradrenaline at day 2 (221±5.1 beats/min) was significantly greater than that at days

<table>
<thead>
<tr>
<th>Day</th>
<th>Noradrenaline (×10^-7 M)</th>
<th>Tyramine (×10^-5 M)</th>
<th>Acetylcholine (×10^-5 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4.05±0.72 (14)</td>
<td>1.09±0.15 (7)</td>
<td>1.39±0.90 (15)</td>
</tr>
<tr>
<td>5</td>
<td>4.01±0.85 (10)</td>
<td>1.36±0.69 (5)</td>
<td>2.33±0.95 (10)</td>
</tr>
<tr>
<td>10</td>
<td>4.66±1.31 (8)</td>
<td>0.90±0.13 (6)</td>
<td>3.10±2.31 (9)</td>
</tr>
<tr>
<td>20-30</td>
<td>4.85±0.64 (13)</td>
<td>1.47±0.33 (10)</td>
<td>6.13±2.06 (12)^a</td>
</tr>
<tr>
<td>60-90</td>
<td>4.08±0.87 (9)</td>
<td>1.66±0.36 (8)</td>
<td>9.90±3.61 (6)^b</td>
</tr>
<tr>
<td>180-210</td>
<td>3.50±1.87 (5)</td>
<td>0.92±0.30 (5)</td>
<td>6.72±3.43 (5)^c</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate the number of preparations.

a, Significantly different from the value at day 2, P<0.05. b, P<0.01.
FIG. 2. Modification by cocaine of the dose-response relationship of noradrenaline in atria from mature and immature rabbits. Average atrial rates in control and cocaine-treated atria and those after wash-out of cocaine (After wash) were 98±9.2, 103±7.9 and 102±8.2 beats/min, respectively (days 60-90, left figure); those were 131±4.7, 135±3.8 and 124±5.4 beats/min, respectively (days 2-12, right figure). Figures in parentheses indicate the number of preparations used. a, Significantly different from respective controls, \( P<0.05 \). b, \( P<0.02 \). c, \( P<0.001 \).

FIG. 3. Dose-chronotropic response curves of tyramine in atria from rabbits at different stages of development. Figures in parentheses indicate the number of preparations used.

FIG. 4. Positive chronotropic response to transmural stimulation of intracardiac adrenergic nerves in atria from rabbits at different stages of development. Absolute values of the atrial rate before the nerve stimulation are presented at the left of the figure. Figures in parentheses indicate the number of preparations used.
Age-dependent chronotropic response

20–30 (205 ± 5.1 beats/min) (P < 0.05), at days 60–90 (195 ± 4.7 beats/min) (P < 0.01) and at days 180–210 (194 ± 8.7 beats/min) (P < 0.02). Median effective concentrations (ED50) in these atria were not appreciably different (Table 1).

Treatment for 20 min with 3 × 10⁻⁶ M cocaine shifted the dose-response curves of noradrenaline to the left in immature (days 2–12) and mature (days 60–90) rabbit atria (Fig. 2). Mean values of ED50 decreased from 6.8 × 10⁻⁷ M to 1.7 × 10⁻⁷ M in immature rabbit atria and from 8.3 × 10⁻⁷ M to 4.8 × 10⁻⁷ M in mature atria. Repeated washing of the prepara-

Fig. 5. Noradrenaline contents in ventricles from rabbits at different stages of development. Vertical bars represent standard errors of means. Figures in parentheses indicate the number of samples. a, Significantly different from the value at day 2, P < 0.001. b, P < 0.01.

Fig. 6. Dose-chronotropic response curves of acetylcholine in atria from rabbits at different stages of development. Values at '0' on the abscissa represent atrial rates before the addition of acetylcholine. Figures in parentheses indicate the number of preparations used.

Fig. 7. Bradycardia induced by transmural stimulation of intracardiac cholinergic nerves in atria from rabbits at different stages of development. Absolute values of the atrial rate were the same as those in Fig. 4 except that the mean rate at day 180 was 86 ± 3.2 beats/min (N = 5). a, Significantly different from the value at day 2, P < 0.05.
Dose-chronotropic response curves of tyramine in atria from different ages of rabbits are shown in Fig. 3. Significant increase in the atrial rate was induced at $5 \times 10^{-6}$ M in these atria. Maximum rate induced by $10^{-4}$ M tyramine was greater at days 2 and 5 than at days 60-90 and days 180-210. ED50's of tyramine are summarized in Table 1 and it can be seen that there is no appreciable difference in atria from rabbits at different stages of development.

Positive chronotropic effect of transmural electrical stimulation was abolished by tetrodotoxin ($10^{-7}$ M) and propranolol ($10^{-6}$ M), and is considered to be the result of release of noradrenaline from adrenergic nerve terminals (11). Increase in the atrial rate induced by the neural stimulation was related directly to age (Fig. 4).

Contents of noradrenaline in ventricles increased with development from day 2 to day 30, and levelled off after day 30 (Fig. 5).

Effects of acetylcholine and transmural stimulation of cholinergic nerves

The addition of acetylcholine ($2 \times 10^{-8}$ to $10^{-5}$ M) caused a dose-dependent decrease in the rate of atria from neonatal, young and adult rabbits (Fig. 6). Significant decrease in the rate was obtained by $5 \times 10^{-7}$ M acetylcholine at days 2 and 5, but by $2 \times 10^{-6}$ M acetylcholine at days 60-90 and 180-210. The ED50 was significantly lower at day 2 than at days 20-30, 60-90 and 180-210 (Table 1).

The negative chronotropic effect of transmural electrical stimulation was abolished by $10^{-7}$ M tetrodotoxin and $10^{-6}$ M atropine. The trends toward increase in the effect of the stimulation at frequencies of 20 and 100/sec were age-dependent (Fig. 7).

DISCUSSION

The highest pacemaker rate of right atria isolated from rabbits was seen at day 2, and this rate gradually slowed with age. Tachycardia observed in neonatal rabbit atria was not altered by propranolol or by reducing the passive tension, therefore the adrenergic beta mechanism and stretching of S–A nodes (12, 13) are not involved. The rate was effectively slowed by acetylcholine (present study) and also by increase in $K^+$, elevation of $Ca^{++}$ or deprivation of $Na^+$ in bathing media (unpublished data). These compounds and procedures are known to increase the membrane permeability for $K^+$, to reduce the $Na^+$ permeability or to decrease the transmembrane influx of $Na^+$. These findings suggest that mechanisms underlying the tachycardia in neonatal rabbit atria are related to membrane permeability for ions of S–A nodal pacemaker cells, and that this permeability is quite different from that in adult rabbit atria.

Since the atrial rate under steady state conditions was markedly different, it was difficult to compare the positive chronotropic effect of noradrenaline in atria from rabbits of different ages. However, as far as concentrations sufficient to induce a significant increase in the rate and ED50's are concerned, the responses to the amine did not differ in these atria. This is not in agreement with data obtained from dogs and rats that the heart of infant animals in situ is more sensitive to catecholamines (1, 2) and also with the opposite results.
AGE-DEPENDENT CHRONOTROPIC RESPONSE

in rats that the adult heart is more sensitive to noradrenaline (3). Whether or not these discrepancies are due to differences in species and in experimental conditions remains the subject of further study.

It has been demonstrated that adrenergic innervation to the heart develops after birth (5) and the re-uptake of noradrenaline by the nerves is less in neonatal and young rabbit hearts than in adult hearts (14, 15). In the present study, however, similar potentiation of the effect of noradrenaline in immature (days 2–12) and mature (days 60–90) rabbit atria by cocaine was observed. The potentiating effect of cocaine in the heart is due mainly to the inhibition of re-uptake of the amine (16). Therefore, it appears that the neuronal re-uptake mechanism plays a significant role in inactivating noradrenaline in immature rabbit atria as with mature atria.

Findings obtained in the present study that content of noradrenaline in rabbit ventricles increased significantly with development from day 2 to day 30 are quite consistent with the result seen in rabbit whole hearts (5). In addition to increasing the accumulating ability of noradrenaline in adrenergic nerves (14, 15), the nerve density in the myocardial wall may also increase with development of the heart. In their fluorescence histochemical study, Friedman et al. (5) suggested that a significant proportion of noradrenaline measured in the newborn heart resides in the preterminal nerve trunks and therefore may not be in close anatomical proximity to adrenergic receptors in the myocardial cell. In the present study, however, the response to tyramine was not appreciably altered with development. Tachycardia induced by electrical stimulation of adrenergic nerves increased with development of atria, however, such an apparent age-dependent difference in the response may be related to the atrial rate under steady state conditions which is markedly different in neonatal, young and adult rabbit atria. Only a small fraction of the cardiac store of noradrenaline is required to elicit functional responses to tyramine (17) or adrenergic nerve stimulation (18). Therefore, it appears that functionally available noradrenaline released by chemical or electrical stimulation is not appreciably different in atria from rabbits at different stages of development.

It has been demonstrated that the negative inotropic effect of acetylcholine on isolated atrial myocardium is similar in the fetal lamb (at the last 15 days of gestation) when compared with the adult sheep (19). However, in the present study, the negative chronotropic effect of acetylcholine was significantly greater in neonatal and young rabbit atria than in those of adult rabbit atria. In contrast, the chronotropic response to cholinergic nerve stimulation at high frequencies (20 and 100/sec) increased with age and such data are consistent with the result observed in in situ experiments (7). These findings suggest that the transmitter release by cholinergic nerve stimulation increases with development of atria.

The tachycardia in neonatal rabbit atria may be due to different electrogenic characteristics of pacemaker cells from those in adult rabbit atria and cholinergic nerves innervating the S-A node may develop postnatally. On the other hand, our data strongly suggest that adrenergic nerve fibers in rabbits develop at an early postnatal stage and responsiveness to chemical or electrical stimulation of adrenergic nerves and to noradrenaline is already
established in neonatal rabbit S-A node.

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REFERENCES

2) ADOLPH, E.F.: Am. J. Physiol. 209, 1095 (1965)
4) BAUER, D.J.: J. Physiol. 95, 197 (1939)
6) BLOOR, C.: J. Physiol. 174, 136 (1964)
8) WEST, T.C. AND TODA, N.: Circulation Res. 20, 18 (1967)
19) FRIEDMAN, W.F.: Prog. Cardiovascular Dis. 15, 87 (1972)