Quantitative evaluation of ontogenetic change in heart rate and its autonomic regulation in newborn mice with the use of a noninvasive piezoelectric sensor

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Sato S. Quantitative evaluation of ontogenetic change in heart rate and its autonomic regulation in newborn mice with the use of a noninvasive piezoelectric sensor. Am J Physiol Heart Circ Physiol 294: H1708–H1715, 2008. First published February 8, 2008; doi:10.1152/ajpheart.01122.2007.—A reliable basal heart rate (HR) measurement in freely moving newborn mice was accomplished for the first time by using a novel noninvasive piezoelectric transducer (PZT) sensor. The basal HR was about 320 beats/min at postnatal day (P)0 and increased with age to about 690 beats/min at P14. Contribution of autonomic control to HR was then assessed. Sympathetic blockade with metoprolol significantly reduced basal HR at both P6 (236 ± 23 beats/min; mean ± SE) and P12 (105 ± 8 beats/min), but atropine was without effect, indicating the predominant tonic adrenergic stimulation and absence of vagal control for basal HR in newborn mice. In contrast to stable basal HR during 5-min recording, HR measured by ECG (ECG-HR) was markedly decreased because of the restraint stress of attaching ECG electrodes, with accompanying freezing behavior. ECG-HR lowered and further decreased gradually during 5 min (slow cardiodeceleration) at P0–P3 and rapidly decreased and gradually recovered within 5 min (transient bradycardia) at P9–P14. The response was not uniform in P4–P8 mice: they showed either of these two patterns or sustained bradycardia (9–29%), and the number of mice that showed transient bradycardia increased with age (30–100%) during the period. Studies with autonomic blockade suggest that the slow cardiodeceleration and transient bradycardia are mediated mainly by withdrawal of adrenergic stimulation and phasic vagal activation, respectively, and the autonomic control of HR response to restraint stress is likely to change from the withdrawal of adrenergic stimulation to the phasic vagal activation at different stages during P4–P8 in individual mice. The PZT sensor may offer excellent opportunities to monitor basal HR of small animals noninvasively.

slow cardiodeceleration; circulating catecholamine; phasic vagal activation; transient bradycardia; baroreflex

These developmental changes in HR are likely to depend largely on intrinsic cardiac pacemaking. In addition, the susceptibility to extrinsic influences such as sympathetic and parasympathetic nerve stimulation of the heart, catecholamine release from the adrenal medulla, and other humoral factors also contribute to the ontogenetic change in HR. It has been reported that tonic autonomic influences on the heart undergo extensive maturational change during the first few postnatal weeks in rats (1, 2, 3, 15, 22, 26, 36–38). The general principle is that tonic activity of the sympathetic nervous system influences basal HR earlier in development and activity of the parasympathetic nervous system emerges later, although the precise ages at which the sympathetic and parasympathetic influences first emerge has not been fully established.

It should be noted, however, that HR has been evaluated by using ECG with subcutaneous electrodes in many studies, and therefore the measured HR does not necessarily represent the basal HR under nonstressed conditions, particularly in small animals. This is because stressor stimuli due to the attachment of ECG electrodes produce a sympathetically and/or parasympathetically mediated response in HR. It has been shown that there is a series of stages by which HR responsiveness to stressor stimuli becomes established during the first few postnatal weeks (7, 15, 16, 18, 23, 31). According to the pioneering work by Hofer and Reiser in 1969 (15), HR is unresponsive to stressor stimuli in the first few days but decreases between 6 and 16 days of age. Finally at 20 days, the stressor stimuli are associated with an increase in HR (15), the adult pattern for most species. In this respect, noninvasive measurement of HR is a prerequisite for describing and comparing the basal HR during the first few postnatal weeks. Recent advances in telemetry technology may offer the possibility of an accurate, reliable, and less invasive recording of ECG in unrestrained animals (8, 39). However, this requires surgical implantation of telemetry devices, and therefore possible interference from surgical stress cannot be completely excluded, particularly in small animals during the early postnatal period.

I have recently developed a novel system for monitoring HR in mice with a piezoelectric transducer (PZT). The device detects heartbeat vibration and converts it into an electrical signal with a custom-designed analog circuitry and a microprocessor program (Ref. 34; US Patent No. 7174854). With this device, noninvasive measurement of HR (PZT-HR) is accomplished simply by putting a newborn mouse on the sensor of the PZT system. In the present study, the ontogenetic change in HR in mice was measured noninvasively from the fetal period to the early postnatal period. For example, the human HR is about 140 beats/min at birth and then decreases gradually during the course of development to about 70 beats/min in adults. Animals such as lambs (5, 6, 35) and pigs (12, 41) also show qualitatively similar ontogenetic changes in HR. On the other hand, the developmental change in HR follows a different time course in small animals. In rats, HR increases from about 300 beats/min at birth to about 500 beats/min at about 1 mo of age (15, 37). HR of anesthetized newborn mice (rats) also increases from about 360 (±45) to 440 (±490) beats/min during the first postnatal week (17, 26).

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MATERIALS AND METHODS

One hundred sixty-two C57BL/6J mouse pups aged 0–14 days born in our laboratory were raised in a litter with their dam in a home cage 20 cm long, 12 cm wide, and 11 cm high. Pregnant C57BL/6J mice were monitored by an infrared camera (MK-0323E; Akizuki, Japan) from a week before parturition and recorded by a DVD recorder (DVR-530H, Pioneer) to obtain the exact delivery time. The pups with their dam were housed at ±25°C under a 12:12-h light-dark regimen with food and water provided ad libitum, and mice were euthanized on P14 by CO2 inhalation after the experiments, in accordance with the protocol approved by the Akita University Institutional Committee for Animal Studies.

Heart Sound Detection by PZT Sensor

The method for detecting heart sounds with the PZT system was described previously (34). Because the PZT system (ATC-402, Unique Medical), which consisted of a heater-controlled PZT sensor and a main unit, was designed for use in adult mice, the PZT sensor in the present study was modified to increase its sensitivity. The PZT sensor was constructed simply by mounting a naked PZT [disk shaped, 35-mm outer diameter (OD), EE35A-30A, FDK] on a copper plate (70 × 130 × 1 mm) with four small rubbers (2 mm thick) and then placing it on the heater-controlled PZT sensor, which was used as a heater device only for temperature control. Second, an additional amplifier (gain 40 dB) was added to amplify the weak PZT sensor output signal (PZT signal; see Fig. 1). Furthermore, a handmade heart sound detector (HSD) circuit (34) was connected to the PZT sensor output for the recording of heart sound detecting signal (HSD signal) and digital output (HSD output) because the ATC-402 has a HSD circuit but not output terminals for these signals.

Temperature Control

Temperature control is crucial for measuring HR. Therefore, I explored the optimum PZT surface temperature by measuring the body surface temperature of P6 mice placed on the PZT with different surface temperatures of 25, 31, and 35°C at 0 and 5 min after being picked up from the home cage. The body surface temperature, which was measured by holding a noncontacting infrared digital thermometer (MESI-1, Medical Electronics Science Laboratory) very close to the back of the mouse (<5 mm), slightly increased (0.8 ± 0.2°C, mean ± SD; P = 0.011, n = 6, Student’s paired t-test) at the PZT surface temperature of 31°C, while it decreased (−2.0 ± 0.3°C, P = 0.002, n = 6) and increased (1.7 ± 0.2°C, P = 0.001, n = 6) at 25 and 35°C, respectively. The change in HR in the mice during 5 min was not significant at a PZT surface temperature of 31°C (−4 ± 4 beats/min; P = 0.33, n = 11), while it significantly decreased at a temperature of 35°C (−80 ± 16 beats/min; P = 0.005, n = 6). Thus the PZT surface temperature was set at 31°C, and experiments were performed at a room temperature of 25.3 ± 0.4°C (n = 39) in the present study. The surface temperature of the PZT, measured by a thin thermocouple probe of 0.3-mm OD (PTC-201, Unique Medical), was 30.9 ± 0.2°C (n = 8) and 33.3 ± 0.4°C (n = 5) during the absence and presence of mice on the PZT, respectively, when the temperature of the ATC-402 heater device was adjusted to 34°C.

PZT-HR Measurement

Because picking up all the pups from each litter every day often made their dam nervous enough to abandon nursing and eat them all, I recording for each litter was not performed every day but on several days during the first 14 postnatal days and 2) no more than three mice from one litter were randomly chosen, regardless of sex, for most recordings. Mice were not subjected to sex selection because it was hardly possible to distinguish sex. To avoid the interference of circadian rhythm with basal HR, recording of heart sounds with the

![Fig. 1. A: a representative piezoelectric transducer (PZT) recording in postnatal day (P)12 mice. From top to bottom, PZT signal, heart sounds, heart sound detector (HSD) signal, HSD output, ECG, and breathing movement signal are shown. B: representative traces recorded at P0 in 3 different mice. Top: 2 S1 [in heart sounds (arrow), in HSD signals (○)] and 3 S2-like signals (▼) were observed. Middle: S1 was predominantly observed in the PZT signal (▼) and HSD signal (○), not in the heart sounds. Bottom: tiny S1 signals (arrow) corresponded to R waves in ECG.](http://ajpheart.physiology.org/DownloadedFrom)
PZT system (PZT recording) and ECG recording were performed between 830 and 1900 in 172 of 178 recordings. Because the remaining six pups were born at either 1530 or 830, their recordings were performed at 2020 or 350, so as to obtain the HR at 12 h (P0.5) after birth. All recordings were performed with a delay of 0.5–3 h, 0–1 h, and 1–4 h in P0, P0.5–P8, and P9–P14 pups, respectively. Accordingly, the basal HR data were obtained almost punctually at an interval of 24 h over the 2 wk.

For the PZT recording, pups at rest or sleep were transported ~110 cm from their home cage onto the PZT sensor in 2 s by holding their neck with the thumb and forefinger. The PZT recordings were then started within several seconds, except for the pups that continued exploring the novel environment. In the latter case, the start of the PZT recording was delayed for no more than 1 min, until the pups rested or slept after the active state. In addition, a plastic ring of 42-mm inner diameter and 29-mm height was placed around the PZT so that a mouse on the PZT could not escape. The PZT sensor was placed on a pile of paper towels (Kimtowels; Nippon Paper Crecia) placed on a conventional steel desk to reduce the vibration noise from the surroundings.

The pups underwent PZT recording for 5 min followed by an ECG recording for 5 min (Fig. 2), for the later comparison of PZT-HR as a basal HR with HR obtained from ECG (ECG-HR).

**ECG-HR Measurement**

ECG electrodes were made of J-shaped (hook) gold-plated steel wires of 0.4-mm OD that were connected with 1.5-mm-OD shielded wires (ATC-401EM-A, Unitec Medical). For the two-lead ECG recording, a mouse was picked up and moved ~3 cm to the side of the heater device on the paper towel and the ECG electrodes were hooked around each of three limbs and tightened with rubber stoppers (5.0-mm OD × 2.3-mm thickness), using tweezers for less pain. The mouse was then replaced on the PZT sensor without the plastic ring. Although attaching the ECG electrodes inevitably led to the mice struggling for several seconds, most of them quickly stopped their motion and exhibited immobility thereafter. Therefore, the ECG recording accompanied by the PZT recording was available for the ECG-HR measurement.

**Pharmacological Study**

After the 5-min PZT recording for basal HR measurement, the muscarinic receptor blocker atropine, the postsynaptic β-adrenergic receptor blocker metoprolol, or both (dual blockade) were administered to the pups at P6 and P12 to determine the sympathetic and parasympathetic contributions to the basal HR during the neonatal period. Atropine sulfate (Sigma) and metoprolol tartrate (MP Biomedical) were dissolved in 0.9% saline solution before the experiments, and a 30-gauge insulin syringe (U-100; Becton-Dickinson) was used for intraperitoneal injection (2 mg/kg) into P6 and P12 pups weighing ~3 and ~5 g, respectively. The dose of drugs was chosen on the basis of previous studies (2, 15, 39). After these autonomic blocker injections, the pups were again placed on the PZT sensor for 10 min and postblockade PZT-HR was recorded for 5 min. After this, ECG-HR was recorded for 5 min to assess the autonomic control of HR response to ECG electrode attachment (Fig. 2). Additional P4 and P14 mice were subjected to atropine administration to assess the parasympathetic contribution to the HR response in more detail.

**Data Acquisition and Analysis**

All output from the PZT system including buffered PZT signal, heart sounds, breathing movement signal, HSD signal and HSD output, and ECG (Fig. 1A) were stored in a computer by Clampex9 software with an analog-to-digital converter (digidata1322A, Molecular Devices) at a sampling interval of 200 μs. PZT-HR was calculated by averaging 10 (or <10 in mice with weak heart sounds or vigorous activity) successive intervals of the first heart sound (S1-S1 interval). The S1-S1 intervals in P0–P2 mice were carefully determined by observing the weak and inarticulate traces of PZT signal, heart sounds, and HSD signal, which were displayed on the computer screen by analysis software (Clampht9, Molecular Devices; see Fig. 1B). ECG-HR was also calculated by averaging 10 successive R-R intervals.

The following data were removed from analysis for estimation of the basal HR: 1) data from mice of extremely low weight (n = 3) or whose mother died (n = 3); 2) data from mice with severe bradycardia during the PZT-HR measurement (n = 6); and 3) PZT-HR data with HR change during 5 min [ΔHR(5 min) = HR(5 min) − HR(0 min)] beyond mean ΔHR(5 min) ± 2SD of each age group (n = 12).

Data are expressed as means ± SD unless otherwise stated. Statistical significance between two groups was assessed depending on the homogeneity of the variance by Student’s paired t-test or by Welch’s t-test. For multiple comparisons, post hoc Dunnett’s test or Tukey’s test was used as appropriate after the application of one-way analysis of variance (ANOVA) with Bartlett’s test. Mann-Whitney U-test was applied in cases in which the assumption of the homogeneity of variance for a multiple comparison was violated or the numbers of data in a pair were different.

**RESULTS**

**Basal HR**

Representative recording from a P12 mouse is shown in Fig. 1A. The PZT signal (top trace) has mixed components of breathing movement and heart sounds. The heart sounds were clearly isolated (2nd trace) by a band-pass filter from the PZT signal. The first (S1) and second (S2) sounds were identified as tiny, regular heart sounds (Fig. 1B) in the raw PZT signal and not in the heart sounds (Fig. 1B, middle). Alternatively, S1 signals corresponding to R waves in the ECG were identified as tiny, regular fluctuations in the heart sounds (Fig. 1B, bottom). In the

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**Fig. 2.** Diagram of experimental protocol. PZT heart rate (PZT-HR) recording was followed by ECG recording under control condition (no drugs) and autonomic blockade. Intraperitoneal drug injection and ECG electrode attachment took <1 min in most cases. Postdrug PZT recording was started at 10 min after drug administration.
present study, $S_1$–$S_1$ intervals were carefully determined in P0–P2 mice by checking the raw PZT signal, heart sounds, and HS D signal, and several to 10 successive $S_1$ were used to calculate PZT-HR.

Basal HR (PZT-HR) increased in an exponential-like curve from 323 ± 38 beats/min at P0 to 692 ± 40 beats/min at P14, which is comparable to the HR of adult conscious C57BL/6 mice (539–724 beats/min) (8, 11, 20, 40). The basal HR remained unchanged during 5-min recordings at each age (Fig. 3A).

Autonomic Control of Basal HR

The contribution of sympathetic and parasympathetic control to basal HR was examined in mice at P6 ($n = 6$) and P12 ($n = 5$) (Fig. 3, B and C). Atropine administration (2 mg/kg ip) did not significantly change the PZT-HR at P6 (−34 ± 12 beats/min, mean ± SE; $P = 0.099$, ANOVA with post hoc Dunnett’s test) and P12 (−9 ± 12 beats/min, $P = 0.700$), respectively, compared with the response to saline injection. On the other hand, metoprolol administration (2 mg/kg ip) markedly decreased the PZT-HR by −236 ± 23 beats/min ($P = 0.000$) and −105 ± 8 beats/min ($P = 0.005$) at P6 and P12, respectively. Similarly, dual autonomic blockade significantly decreased the PZT-HR by −242 ± 20 beats/min ($P = 0.000$) at P6 and −182 ± 17 beats/min ($P = 0.000$) at P12.

HR Response to ECG Electrode Attachment

It was noted that PZT-HR was stable during the 5-min measurement, and the following attachment of ECG electrodes caused a variable HR response that depended on postnatal day. Figure 4, A and B, show representative traces of PZT-HR and following ECG-HR recorded from P2 and P12 mice, respectively. At P2, HR decreased gradually after the attachment of ECG electrodes. On the other hand, HR suddenly dropped on ECG electrode attachment at P12 and recovered gradually during the 5-min recording. It was also confirmed that PZT-HR of P2 mice remained unchanged for 10 min (0.5 ± 5.8 beats/min, mean ± SD; $P = 0.93$, $n = 8$) (Fig. 4C). Figure 5, A–C, summarize the changes in PZT-HR and ECG-HR during 5 min measurement at P2, P6, and P12, respectively. These three age-dependent ECG-HR responses were tentatively classified into the following three patterns: slow cardiodeceleration (Fig. 5A), which showed a gradual decrease in HR at almost constant decreasing rate during 5 min; sustained bradycardia (Fig. 5B), which showed a rapid decrease in HR at 0 min and maintained it for 5 min; and transient bradycardia (Fig. 5C), which showed a rapid increase in HR at 0 min and its gradual recovery within 5 min.

Change in HR after ECG electrode attachment. The initial response of HR to the attachment of ECG electrodes is summarized in Fig. 6A. The difference between the PZT-HR at 5 min and the ECG-HR at 0 min was not significant at P0 ($P = 0.360$) but became significant at P0.5 (−8 ± 7 beats/min; $P = 0.039$), and it reached a maximum decrease of −163 ± 25 beats/min ($P < 0.000$) at P11 (Fig. 6A). It should also be noted that the attachment of the ECG electrodes caused characteristic behavioral responses in all mice examined. The mice struggled upon attachment of the electrodes, but after several seconds they stopped their motion and exhibited freezing behavior. This was a consistent finding irrespective of postnatal day. Figure 6B shows three age-dependent stages of HR response during the first two postnatal weeks: slow cardiodeceleration at P0–P3 ($P < 0.05$), sustained bradycardia at P4–P7 ($P > 0.05$), and transient bradycardia at P8–P14 ($P < 0.05$).

In light of the above findings, I hypothesized that the HR response to ECG electrode attachment changed from slow cardiodeceleration to transient bradycardia during the 14 days after birth and the transition occurred between P4 and P7 (Fig. 6B).
**Individual HR response to ECG electrode attachment.** To verify the hypothesis, individual HR data during 5-min PZT and ECG recording at P6 and P12 were further analyzed (Fig. 7). PZT-HR of individual P12 mice was similar and stable for 5 min at P6 (Fig. 7A, left) and P12 (Fig. 7B, left). ECG-HR of individual P12 mice also showed a similar time course of transient bradycardia (Fig. 7B, right). However, individual P6 mice showed diverse patterns of change in ECG-HR (Fig. 7A, right). The scatter plot in Fig. 7C summarizes the ECG-HR changes in individual P0–P14 mice over 5 min (ΔHR_{min} = HR (5 min) – HR (0 min)). The positive and negative plots indicate transient bradycardia and slow cardiodeceleration, respectively, and plots near zero represent sustained bradycardia as shown in Fig. 7A, right. It appears that the HR response to ECG electrode attachment emerged first as slow cardiodeceleration from birth until P8 and secondary transient bradycardia emerged at P4 and predominated day by day.

Figure 7D illustrates the percentage of mice showing slow cardiodeceleration, transient bradycardia, and sustained bradycardia at each age. The range of sustained bradycardia was heuristically determined as an HR change less than ±5, ±10, and ±30 beats/min for the mice at P0–P1, P2–P7, and P8–P14, respectively. It is clearly demonstrated that the percentage of mice showing transient bradycardia increased with age after its appearance at P4 and became predominant after P9.

**Autonomic control of HR response to ECG electrode attachment.** Figure 8A shows HR responses to ECG electrode attachment at P4, P6, P12, and P14 and those under parasympathetic blockade by atropine. It is clearly shown that the parasympathetic blockade eliminated the transient bradycardia (i.e., all positive plots disappeared) at P4 (Mann-Whitney U-test, P = 0.000) and P6 (P = 0.007), while it diminished at P12 (P = 0.019) and P14 (P = 0.003). This indicates that the transient bradycardia is not merely a fluctuation of HR but is controlled by autonomic nervous system, i.e., it may be mediated by transient vagal control of HR.

In mice at P6, significant slow cardiodeceleration was observed under atropine (−67 ± 11 beats/min, mean ± SE; P = 0.002, Student’s paired t-test), metoprolol (−30 ± 8 beats/min, P = 0.016), and dual blocker (−23 ± 4 beats/min, P = 0.003) administration (Fig. 8B). The slow cardiodecelerations under
metoprolol and dual blockade were significantly smaller than that under atropine administration [45% (P = 0.032) and 33% (P = 0.010), respectively, n = 6; ANOVA with post hoc Tukey’s test]. These results suggest that the slow cardiodeceleration is mediated by sympathetic regulation at least in part.

On the other hand, P12 mice showed transient bradycardia during ECG recording with HR recovery of 120 ± 27, 39 ± 8, and 73 ± 19 beats/min under saline, atropine, and metoprolol injection, respectively, and it was almost completely eliminated by dual blockade (−3 ± 5 beats/min) (Fig. 8C). The magnitude of the transient bradycardia was significantly smaller under atropine (33%, P = 0.047) and dual blockade (0%, P = 0.009) compared with that under saline administration (n = 5, ANOVA with post hoc Dunnett’s test).

**DISCUSSION**

**Basal HR of Newborn Mice**

In an earlier study, newborn mice were held by a rubber glove with small plate electrodes attached to its fingers for ECG recording (4), which, however, showed considerably lower HR than in the present study. The stress caused by the handling procedure (15), i.e., holding with the rubber glove, is likely to have lowered the HR in the previous study. By contrast, the PZT recording in the present study seems to provide a reliable HR measurement. In the PZT recording, grasping the mouse pups by their neck seemed to be less alarming, since most of the pups stayed calm on the PZT, possibly because they were accustomed to being picked up in this way by their dam. Only 19 of 178 mice exhibited marked bradycardia, slow cardiodeceleration, or transient bradycardia after the transfer from the home cage to the PZT sensor. Accordingly, ~90% of PZT-HR measurement was successfully noninvasive without being affected by the handling procedure and the new environment. Therefore, the PZT-HR is likely to represent the basal HR.

**Adrenergic and Parasympathetic Contributions to Basal HR**

Intrinsic HR of mice under dual blockade increases with age from ~340 (P6) to ~480 (P12) beats/min (Fig. 3, B and C) and to ~510 beats/min in adults (19). The intrinsic HR is likely to be further greatly elevated by tonic adrenergic stimulation up to the basal HR (Fig. 3), which is supported by the previous findings, e.g., functional sympathetic innervation in rat heart at birth (1, 2, 28), neurally controlled catecholamine release from adrenal in P2 rat (37), and a high catecholamine concentration produced by a surge of sympathoadrenal activity associated with vaginal delivery (25) and in the umbilical cord blood of neonates (24). On the other hand, parasympathetic blockade with atropine failed to affect the basal HR (Fig. 3, B and C), consistent with a result in adult mice (39) and the days at which tonic parasympathetic tone first emerges in rats, P20 (21) or P24 (37). Accordingly, the basal HR of newborn mice may be
predominantly controlled by the adrenergic stimulation, in contrast to parasympathetic nervous activity, which seems negligible.

HR Response to ECG Electrode Attachment

**Transient bradycardia.** Analysis of HR responses to ECG electrode attachment in individual mice revealed that the phasic vagal control of HR may emerge first at no later than P4 (Fig. 7C), consistent with the appearance of acetylcholinesterase activity in cardiac tissue of rats at P4 (27). This finding would have been overlooked if the HR changes had been evaluated only with averaged values (see Fig. 6B: the transient bradycardia seems to emerge first at P8). On the other hand, the accumulated number of mice that showed transient bradycardia during P4–P8 (Fig. 7D) indicates the change in the HR response from slow cardiodeceleration to transient bradycardia with age, as well as the change from withdrawal of adrenergic stimulation to phasic vagal activation. This is consistent with a rat study demonstrating that the autonomic control of HR progresses from sympathetic control during the first postnatal week to parasympathetic inhibition at weaning (37). Different rates of individual maturation in sensory input, afferent and parasympathetic efferent pathways, central autonomic nervous system, and the cardiovascular system may be the cause of the different days of the transition in individual mice (Fig. 7A, right).

Although the parasympathetic contribution may predominate, the transient bradycardia may be involved with a baroreflex response mediated by both branches of the autonomic nervous system (Fig. 8C), consistent with studies in P6 rats (29, 30), dogs (9), and other species (13). In addition, previous findings of abrupt maturational change in the baroreflex system in P7–P14 mice (17) and the baroreceptor-mediated vagal reflex function in P6 rats (21, 29) all support the present hypothesis that parasympathetic regulation of HR emerges and matures between P4 and P8 in mice.

**Slow cardiodeceleration.** Is the slow cardiodeceleration a real HR response? It is unlikely that the dose of drugs was insufficient for complete abolishment of slow cardiodeceleration in P6 mice, because it was completely abolished at P12 (Fig. 8C) and the result under dual blockade at 10-fold dose (20 mg/kg) was almost identical (−26 ± 20 beats/min, mean ± SD; n = 6) to the result shown in Fig. 8B. On the other hand, it was confirmed that the heater temperature of 31°C in a room at 25°C made both body temperature and HR stable for >10 min in the present study (Fig. 4C). Thirty-minute PZT-HR measurement in P3 mice also showed stable HR and body surface temperature during between 20 and 30 min (difference: 1 ± 5 beats/min, P = 0.57; 0.2 ± 0.2°C, P = 0.11; n = 5). Therefore, it also is unlikely that the long time of measurement (~25 min) is responsible for the slow cardiodeceleration. I consider that the slow cardiodeceleration is a HR response induced by the ECG electrode attachment.

Slow cardiodeceleration clearly appeared under atropine administration (Fig. 8B) in P6 mice but not under saline and control conditions (see Fig. 6B). Because data are averaged, the slow cardiodeceleration of some mice may be cancelled by the transient bradycardia of other mice (see Figs. 6B and 7A, right), while interruption of the canceling by atropine may disclose the slow cardiodeceleration. Withdrawal of catecholamine release from adrenal medulla (36, 37) and/or other organs should lead to slow decrease in circulating catecholamines and therefore slow decrease in HR. In addition, if metoprolol completely blocked the postsynaptic β-receptors or norepinephrine release from cardiac sympathetic nerve terminals was not functional, the slow cardiodeceleration that emerged under dual blockade (Fig. 8B), which further decreased from the HR equal to intrinsic HR, might be produced by a mechanism different from adrenergic control, e.g., humoral factors other than circulating catecholamines.

**Sustained bradycardia.** Sustained bradycardia in P4–P7 mice (Fig. 6B) may be produced partly by the masking between the slow cardiodeceleration and the transient bradycardia as discussed above. Initial decrease in ECG-HR (Fig. 6A) in mice at the first postnatal week may be sustained for 5 min because both ECG-HR of P6 mice at 0 min of the slow cardiodeceleration group and that at 5 min of the transient bradycardia group (Fig. 7A, right) were lower than the preceding PZT-HR at 5 min by 40 ± 15 (n = 5) and 83 ± 27 (n = 6) beats/min, respectively. The sustained bradycardia, which rapidly decreases after the ECG electrode attachment, may be mediated by the withdrawal of neurally controlled adrenergic stimulation of the heart, because it is known that HR reaches a maximum after 10 s of cardiac nerve stimulation in rats (1).

**Freezing behavior induced by ECG electrode attachment.** The freezing behavior found in the present study is likely to be the phenomenon generally known as tonic immobility or feigning death, which a prey exhibits as a defense reaction when approached or restrained by a predator (33). Opossums (10), newborn deer calves (7), deer fawns (18), and deer mice (32) decrease their HR during freezing behavior. Because the ECG electrode wire is hard for small newborn mice, attaching ECG electrodes might be a restraint stress as alarming as being grasped and restrained by a predator.
Conclusions

The present study revealed that the restraint stress due to the ECG electrode attachment leads newborn mice to feign death, which activates or deactivates the autonomic nervous system and elicits various patterns of HR response during the early postnatal weeks. The HR response of the slow cardiodeceleration and the sustained bradycardia seems to be mediated mainly by the withdrawal of circulating catecholamines and sympathetic-neural control of the heart, respectively, while the transient bradycardia may be mediated mainly by phasic vagal activation. Although the involvement of sympathetic neural control with adrenergic stimulation and that of baroreflex with transient bradycardia remain to be clarified, the present results may provide fundamental data for further studies with the use of genetic manipulation techniques, which may clarify details in the mechanisms of developmental changes in autonomic cardiac control during the early postnatal period in small animals.

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