The Utility of the Small Rodent Electrocardiogram in Toxicology

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Extensive research has lead to a growing appreciation that the heart is acutely sensitive to a broad array of toxicants via multiple routes of exposure. These agents are as diverse as the antineoplastic drug doxorubicin and environmental agents including ambient air pollution. Adverse effects in the heart often manifest as a change in the electrocardiogram (ECG). The ECG has long been used in the clinic to assess human cardiovascular health. Surface electrocardiographic recordings (i.e., those made from the skin) in humans often help to detect abnormal myocardial impulse formation, conduction, cardiac rhythm disturbances, and altered autonomic regulation of the heart. In toxicology, the ECG provides a collection of end points that may be used to assess both the quality and magnitude of cardiac toxicity. Increasingly over the last two decades, the cardiotoxicity of agents have been characterized using small rodent electrocardiography. Additionally, tremendous insight into possible mechanisms of action of known human cardiotoxins has been gained. Rat and mouse models offer a number of advantages relative to larger animals including lower cost, less variability, the availability of transgenic models, and a plethora of research tools. Modern day advances in small rodent electrocardiography have enabled assessments in conscious unrestrained animals and improved ECG interpretation. Thus, the incorporation of small rodent electrocardiographic assessments into toxicology studies may facilitate the screening of cardiotoxic potential and the elucidation of mechanisms of action. This review will discuss the utility of the small rodent ECG, various methodologies used to derive ECG data in rats and mice, and various applications in toxicology.

Key Words: electrocardiogram; rat, mouse; toxicology; heart rate variability; arrhythmia.

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BASIC ELECTROPHYSIOLOGY AND THE BASIS OF THE SURFACE ECG IN HUMANS

The ECG is a temporal record of the changes in electrical charge of all four chambers of the heart during each beat. These recordings are obtained in humans using sensing electrodes that are strategically placed on the body’s skin surface and register electrical potential differences stemming from electrical current flow through the heart muscle. Changes in these potentials form the basis for changes in the ECG. Ionic current flows from cell to cell through the heart as a result of the activity of selectively permeable ion channels, which when activated, transiently open allowing the movement of positively charged ions (Na\(^{+}\), K\(^{+}\), and Ca\(^{2+}\)) across a muscle membrane that is otherwise impermeable to the movement of ions. Flow of these ions across the muscle cell membrane follows electrochemical gradients. These gradients are maintained by specialized transporters driven by the hydrolysis of high-energy phosphates such as adenosine triphosphate (ATP) and include the Na\(^{-}\)-K\(^{+}\) adenosine triphosphatase (ATPase) and active Ca\(^{2+}\) transporters or electrically favored because of the reversal potential, i.e., the Na\(^{-}\)-Ca\(^{2+}\) exchanger. Under normal physiological conditions, extracellular Na\(^{+}\) and Ca\(^{2+}\) concentrations are much higher than intracellular levels, whereas the opposite is true for K\(^{+}\) (for a detailed review of the role of ion channels in the propagation of electrical current flow through the heart, please refer to the work by Grant, 2009).

The following is a brief description of the ion channels that form the basis for the cardiac muscle action potential (AP) and the ECG in humans. At rest (phase 4 of the AP; top panel in Fig. 1), the muscle cell is polarized, i.e., its intracellular environment is negative with respect to the extracellular environment causing the cell to have a transmembrane gradient in electrical charge. With the appropriate trigger or action potential, Na\(^{+}\) channels activate and Na\(^{+}\) ions move down the concentration gradient into the cell (phase 0), with attendant depolarization of the heart muscle cell membrane, (i.e., a reversal in its electrical polarity). The inside of the muscle cell becomes temporarily positive with respect to its extracellular environment. After reaching peak depolarization, the cell undergoes a short downward spike toward repolarization because of efflux of K\(^{+}\) (phase 1 of AP) that is immediately counterbalanced by influx of Ca\(^{2+}\) through voltage-gated calcium channels that keeps the muscle cell in a depolarized state (plateau phase; phase 2 of AP). The muscle fiber eventually repolarizes after the closing of these Ca\(^{2+}\) channels and continued activity of K\(^{+}\) channels (phase 3) and then returns to the resting membrane potential (phase 4). The cycle repeats with each depolarization.

Whereas the instantaneous distribution of ion concentrations across the myocyte cell membrane form the electrical basis for the potential differences detected by the ECG, the shape and specific landmarks of the electrocardiographic waveform are determined by an ordered and well-defined electrical activation pattern that travels through the heart. Although not a true anatomical syncytium, the heart is arranged in such a way that enables cells to communicate freely. Specialized intercellular junctions called intercalated discs contain gap junctions that provide low-resistance connections between cells, enabling the rapid spread of excitatory current and the AP throughout the heart (Katz, 2000). The ECG is thus an instantaneous record of all the electrical activity occurring at any given time in the heart and not any individual cell. Other factors that influence the shape of the ECG, as depicted below, include electrode placement relative to the anatomical location of the heart (described in subsequent sections), and factors external to the heart such as pulmonary disease, pericardial effusion, or body habitus. The following is a characterization of the ECG as seen with a standard lead II configuration. In humans and all mammals, electrical activation of the cardiac cycle starts in specialized neuronal tissue, the sinoatrial (SA) node (Fig. 2A), which is located within the right atrium of the heart and, unlike muscle cells, spontaneously and rhythmically depolarizes (controlled by tonic vagal/parasympathetic stimulation at rest). SA nodal activation triggers depolarizations in neighboring muscle cells causing a wave of depolarization to spread over both atria. The surface ECG records a P wave, a positive pattern.
upward deflection in lead II in a normal heart (Fig. 2B). When the depolarization reaches the atrioventricular (AV) node, which is located near the ostium of the coronary sinus in the right atrium near the right ventricle, the speed of propagation of the activation wavefront slows (Katz, 2000). This has the effect of allowing adequate time for the ventricle to fill during atrial contraction. After the AV node, the current passes through the other specialized nonmuscle tissues of the heart including the His bundle, bundle branches and the Purkinje fibers, which activate the interventricular septum and subsequently the lateral free walls of the left and right ventricles. Although local electrical activation of the AV node, His-bundle and bundle branches can be recorded with intracardiac electrodes, their small overall mass relative to the heart yield no measurable potential on the body surface. As such, the interval between the activation of the AV node and conduction through the His-Purkinje system register only an isoelectric line with no measurable potential, the PR segment. As the electrical wavefront passes through the ventricles, the ECG records Q (short-negative deflection), R (large-positive deflection) and S (negative deflection) waves. The QRS complex occurs contemporaneously with atrial repolarization and consequently precludes direct visualization of the atrial repolarization wave. A short isopotential segment, the ST segment follows the QRS complex and corresponds to the time when most ventricular cells are in phase 2, i.e., the plateau phase of the AP. Repolarization of the ventricles then results in a positive deflection, the T wave. The T wave is generally of lower amplitude than QRS but much broader. The longer duration of the T wave results from the greater duration of the repolarization phase compared with the depolarization phase.

The clinical utility of the ECG is derived from the fact that the spatiotemporal distribution of extracellular voltage as manifest at the body surface is a direct reflection of the spatiotemporal distribution of extracellular voltage of the heart. Because of this direct relationship, we are able to infer changes in intracardiac electrical properties based on changes in the registration of electrical events at the body surface.

**FIG. 2.** (A) Illustrations of the human and rat/mouse hearts highlighting the propagation of the AP wave (large white arrows) from the SA node through the ventricles, accounting for the electrical activity detected by the ECG. Although the shapes of the hearts differ slightly and rodent atria are smaller relative to their ventricles, overall anatomy and flow of AP are similar across human and rodent species. (B) Renderings of the human and rat/mouse ECG. Note the shorter duration and absence of a Q wave and ST segment in the rat/mouse ECG relative to the human ECG. RA, right atrium; RV, right ventricle; LA, left atrium; LV, left ventricle; HB, His bundle; BB, bundle branches; PF, Purkinje fibers; 1, Jones (2008); 2, Farraj et al. (2009); 3, Boulaksil et al. (2010).
Additionally, electrical excitation is coupled to mechanical contraction of the muscle and the pumping of blood by the heart. The influx of Ca\(^{2+}\) during the plateau phase of the AP triggers Ca\(^{2+}\)-induced Ca\(^{2+}\) release from intracellular stores, which subsequently triggers the activation of muscle fiber contraction involving the contractile proteins actin, myosin, tropomyosin, and troponin, and the hydrolysis of ATP. Although excitation-contraction coupling (1:1 relationship) is generally the rule, it cannot be assumed to take place all the time, as happens in disease states. It is also important to remember that the ECG does not measure contraction of the muscle, but only electrical activity of the heart at the level of the skin surface. Nonetheless, the potential utility of the ECG in toxicology stems from its capacity to reflect instantaneous changes in biochemical and metabolic processes that either directly or indirectly affect the biophysical properties of the cardiac cell membrane or channels, or intracellular signaling and ion regulation. Moreover, the ECG provides indirect evidence of structural cardiac changes and remodeling that affect automaticity, impulse propagation and other mechanisms of arrhythmia. Although the ECG is not a direct functional measure, it may be used to infer the anatomical orientation of the heart, disturbances of rhythm and conduction, the presence of ischemic injury (Berne and Levy, 2001), and the influence of altered electrolyte concentrations, drugs, disease, and nutritional deficiencies (Detweiler, 1997). In practice, the ECG is recorded in a number of different settings. ECG monitoring may be passive, as in monitoring after an arrhythmia or therapeutic species as well. Two of the most conspicuous differences between humans and rats/mice are in heart rate and AP duration. Average conscious resting heart rate in humans (70 beats per minute; Sheu and Colecraft, 1997) is much slower than it is in rats (300–500 beats per minute; Farraj et al., 2009; Hofstetter et al., 2006; Tontodonati, et al., 2010) and mice (310–840 beats per minute; Hoyt et al., 2007; Kramer et al., 1993). Additionally, human ventricular AP duration (shorter than the duration of mechanical contraction) is more than five times longer than that of rats/mouse (Curtis et al., 1987). The dog, pig, and rabbit have the greatest degree of resemblance to human cardiac ion channel distribution and function (Gralinski, 2003). These species, like humans, rely on IK\(_s\) (delayed rectifier K\(^{+}\) channel current) as the predominant repolarizing current in the ventricles. Rats and guinea pigs, in contrast, rely on IK\(_{to}\) (transient outward K\(^{+}\) channel current) and IK\(_s\) (slowly activating delayed rectifier K\(^{+}\) channel current), respectively, for ventricular repolarization (guinea pigs lack IK\(_s\); Gralinski, 2003). IK\(_{to}\) serves to accelerate the rate of repolarization; the shortening of the plateau phase of the AP is directly related to the density of IK\(_{to}\) (Fig. 1 compares the relative duration of the ventricular AP of humans, rats, and mice). In addition to human-like ion channel expression, the dog demonstrates human-like respiratory sinus arrhythmia (Shykoff et al., 1991) and RR interval variability (Detweiler, 1997) in its ECG.

COMPARATIVE CARDIAC ANATOMY AND ELECTROPHYSIOLOGY: SMALL RODENTS VERSUS LARGER MAMMALS

Several mammalian species have been used to study ECG effects including the goat, sheep, dog, pig, rabbit, guinea pig, ferret, rat, and mouse (Table 1 compares cardiac anatomy and electrophysiology across species). Whereas the underlying electrophysiology and the biophysical principles are the same in large mammals, including humans, and rodents, there are differences in size, anatomy, channel type and distribution, and cellular ionic regulation. Because rats and mice are quadrupeds, their bodies are parallel to the ground, and consequently, their hearts do not rest on the diaphragm and have more room to move within the pericardial cavity. Rat and mouse hearts also have an ellipsoidal shape (Hoyt et al., 2007) and their atria size relative to their ventricles is smaller than that found in humans (lower panel in Fig. 2A; Wessels and Sedmera, 2003). Rat hearts, unlike those of larger species, do not keep pace with increasing body weight during early growth, and thus have smaller heart-to-body weight ratios (Anderson et al., 2006; Detweiler, 1997). With respect to cardiac innervation, the Purkinje fiber network within the ventricles in rats and mice is limited to the subendocardium similar to the hearts in dogs and humans, and unlike that found in larger mammals such as the horse (Detweiler, 1997). In addition, smaller mammals have more tightly packed Purkinje fibers than larger mammals (Sommer and Johnson, 1968). Gap junction distribution and intercalated disk architecture are similar among rats and humans (Gourdie et al., 1991). The major coronary arteries in rats and mice do not sit on the surface of the heart like in humans, but instead are embedded within the myocardium (Anderson et al., 2006).

There are differences in the contractile properties of the heart as well. With respect to isometric force development, smaller mammals like the rat and mouse have shorter actin-myosin cross-bridge attachment time, which limits the amount of force that can be generated with each contraction, but simultaneously enables high heart rates and high shortening velocity. In contrast, rabbit and human hearts have longer attachment time that increases the economy of force production but decreases maximum heart rate and shortening velocity (Hassenfuss et al., 1991). Finally, in rat and mouse hearts, in contrast to those of larger mammals, increasing AP frequency reduces contractile force of the heart muscle (also known as a negative staircase; Detweiler, 1981).

Several electrophysiological differences exist among laboratory species as well. Two of the most conspicuous differences between humans and rats/mice are in heart rate and AP duration. Average conscious resting heart rate in humans (70 beats per minute; Sheu and Colecraft, 1997) is much slower than it is in rats (300–500 beats per minute; Farraj et al., 2009; Hofstetter et al., 2006; Tontodonati, et al., 2010) and mice (310–840 beats per minute; Hoyt et al., 2007; Kramer et al., 1993). Additionally, human ventricular AP duration (shorter than the duration of mechanical contraction) is more than five times longer than that of rats/mice (Curtis et al., 1987). The dog, pig, and rabbit have the greatest degree of resemblance to human cardiac ion channel distribution and function (Gralinski, 2003). These species, like humans, rely on IK\(_s\) (delayed rectifier K\(^{+}\) channel current) as the predominant repolarizing current in the ventricles. Rats and guinea pigs, in contrast, rely on IK\(_{to}\) (transient outward K\(^{+}\) channel current) and IK\(_s\) (slowly activating delayed rectifier K\(^{+}\) channel current), respectively, for ventricular repolarization (guinea pigs lack IK\(_s\); Gralinski, 2003). IK\(_{to}\) serves to accelerate the rate of repolarization; the shortening of the plateau phase of the AP is directly related to the density of IK\(_{to}\) (Fig. 1 compares the relative duration of the ventricular AP of humans, rats, and mice). In addition to human-like ion channel expression, the dog demonstrates human-like respiratory sinus arrhythmia (Shykoff et al., 1991) and RR interval variability (Detweiler, 1997) in its ECG.
Consequently, the dog has been the preferred species of the pharmaceutical industry to detect the cardiac toxicity of drug candidates or “new chemical entities,” and has proven useful in the identification of drugs that trigger QT interval–related arrhythmias. With respect to regulation of calcium in the rat heart, cellular Ca\(^{2+}\) efflux via Na\(^+\)-Ca\(^{2+}\) exchange occurs during contraction and cellular Ca\(^{2+}\) uptake is favored during rest, whereas in rabbit ventricles, cellular Ca\(^{2+}\) uptake occurs during stimulation and cellular Ca\(^{2+}\) loss occurs during rest (Shattuck and Bers, 1989). Also, the rate of Ca\(^{2+}\) removal, which reflects primarily the Ca\(^{2+}\) extruding activity of the Na\(^+\)–Ca\(^{2+}\) exchanger, is greatest in hamsters followed by guinea pigs, humans, and rats (Sham et al., 1995).

Other unique electrophysiological characteristics of the rat/mouse include electromechanical uncoupling at rapid stimulation rates, a brief QT interval, the absence of a Q wave in most leads of the ECG, the lack of a common isoelectric baseline for the P, QRS, and T waves (Driscoll, 1981) and perhaps most prominently the lack of an isoelectric ST segment (Fig. 2B compares human and rat/mouse ECGs). Humans (and other large mammals) have isoelectric segments in their ECG. Neonatal rats and mice do have distinct isoelectric ST segments but undergo changes with maturity owing to a progressive shortening of the AP. The plateau phase of the AP becomes abbreviated over time because of the emergence of the K\(^{+}\)-carrying IK\(_{to}\) (transient outward current) in the ventricular myocardium (Gussak et al., 2000). Consequently, the beginning of the T wave merges with the end of the QRS complex without an isoelectric ST segment. The absence of clear separation between the QRS complex and the T wave is complicated by the fact that the T wave of the mouse ECG (which is often notched) does not represent complete ventricular repolarization (Danik et al., 2002). Although rats and mice lack an equivalent human ST segment, perturbations that result in ST segment changes in species with ST segments produce a similar shift in the corresponding QRS-T wave region of the ECG in rats (Detweiler, 1981). Some investigators have suggested that normal mouse and rat ventricular repolarization is also characterized by a J wave, which has been identified as the down-sloping portion of the ST segment and is believed to be due to the electrical heterogeneity of the ventricular wall (IK\(_{to}\)-mediated current present in the epicardium, but not endocardium, in rats and mice) (Antzelevitch and Fish, 2001).

Noncardiac characteristics unique to the rat/mouse that may impact cardiac responses include a higher rate of drug metabolism than larger animals necessitating higher doses of drugs to elicit certain effects. Rats, in particular, are susceptible to Pseudomonas aeruginosa lung infections (Curtis and Walker, 1987). Additionally, unlike humans, rats undergo a hypothermic response (reduction in core body temperature, heart rate, and metabolism) when exposed to toxicants, which may confer resistance to the effects of the toxicant (Watkinson et al., 1997). Despite these differences, the use of the rat/mouse to study adverse electrocardiographic effects has advantages over other species including: (1) rats and mice are well standardized and generally have much less interindividual variability than dogs—this includes less variable coronary collateral anatomy, which contributes to more predictable responses to experimental challenges (e.g., acute myocardial ischemia; Curtis et al., 1987; Maxwell et al., 1987); (2) rats and mice are smaller and consequently much easier and less expensive to handle; (3) rats and mice have less potentially confounding preexisting disease, (4) rats and mice exist in multiple disease models or can easily be manipulated to generate such models; (5) rats and mice are available as transgenic models enabling the dissection of various components of the myocardial conduction apparatus and other genes; (6) rats and mice are widely available in large numbers enabling in depth study; and (7) rats and mice have a greater number of species-specific reagents and research tools (Curtis et al., 1987). It is because of such characteristics that the rat/mouse models are often the very first in vivo models that are used early in the drug development process and are also the most commonly used models in basic and applied toxicology studies. Other than differences in heart rate and rate-dependent measured parameters such as PR, QRS, and QT intervals, there

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**TABLE 1**

<p>| Key Electrophysiological Characteristics in Humans and Laboratory Animal Species |
|-----------------------------------|---------------------------------|-----------------|---------------|----------|-------|</p>
<table>
<thead>
<tr>
<th>Human</th>
<th>Dog</th>
<th>Rabbit</th>
<th>Guinea Pig</th>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting heart rate (bpm)</td>
<td>~75(^{e})</td>
<td>~70(^{e})</td>
<td>~200(^{f})</td>
<td>~230(^{e})</td>
<td>~300(^{e})</td>
</tr>
<tr>
<td>Coronary collaterals</td>
<td>Low(^{d})</td>
<td>Middle(^{d})</td>
<td>None(^{d})</td>
<td>High(^{d})</td>
<td>Low(^{d})</td>
</tr>
<tr>
<td>Ventricular AP duration (ms)(^{p})</td>
<td>~250(^{o})</td>
<td>~250(^{o})</td>
<td>~120–140(^{o})</td>
<td>~140(^{o})</td>
<td>50(^{o})</td>
</tr>
<tr>
<td>Primary repolarizing current</td>
<td>IKr,(^{s})</td>
<td>IKr,(^{s})</td>
<td>IKr,(^{s})</td>
<td>IKr,(^{n})</td>
<td>IKr,(^{n})</td>
</tr>
<tr>
<td>Ca(^{2+}) Reg: Na-Ca exchanger activity</td>
<td>Higher(^{r})</td>
<td>?</td>
<td>?</td>
<td>Highest(^{r})</td>
<td>High(^{r})</td>
</tr>
<tr>
<td>Q wave in ECG</td>
<td>Yes(^{s})</td>
<td>Yes(^{s})</td>
<td>Yes(^{s})</td>
<td>Yes(^{s})</td>
<td>No(^{s})</td>
</tr>
<tr>
<td>ST segment in ECG</td>
<td>Yes(^{s})</td>
<td>Yes(^{s})</td>
<td>Yes(^{s})</td>
<td>Yes(^{s})</td>
<td>No(^{s})</td>
</tr>
</tbody>
</table>

Note. AP, action potential; bpm, beats per minute; ms, milliseconds; Ca\(^{2+}\) Reg, Ca\(^{2+}\) regulation.

\(^{a}\)Sheu and Colecraft (1997); \(^{b}\)Dyson et al. (2002); \(^{c}\)Suckow et al. (2002); \(^{d}\)Harkness et al. (2002); \(^{e}\)Kohn and Clifford (2002); \(^{f}\)Jacoby et al. (2002); \(^{g}\)Maxwell et al. (1987); \(^{h}\)Wang et al. (2010); \(^{i}\)Millar and Williams (1982); \(^{j}\)Lemmens-Gruber et al. (1996); \(^{k}\)Spear (1981); \(^{l}\)Liu et al. (2003); \(^{m}\)Gralinski (2003); \(^{n}\)Sham et al. (1995); \(^{o}\)Detweiler (1997); \(^{p}\)Dependent on heart rate; \(^{q}\)Lacks IK\(_{to}\).
is no discernible difference between the rat and mouse ECG. In addition, rats and mice respond similarly to exogenous challenges including ischemic agents (Janse et al., 1998; Wehrens et al., 2000). Although the rat’s larger size facilitates telemeter (ECG transmitter) implantation procedures, mouse telemetry is becoming more common because of the greater availability of mouse-specific reagents and transgenic models compared with rats.

**Human-Rodent Parallels in Cardiotoxicity**

The pathophysiological changes associated with toxicant exposure or disease manifest similarly in the ECG of rodents and humans. For example, the pathological Q wave, a hallmark sign of myocardial necrosis in human myocardial infarction, has been demonstrated in multiple rat models of myocardial infarction and cardiomyopathy (Bestetti and Oliveira, 1990; Carll et al., 2010). Other ECG changes (including P-wave enlargement, prolonged PR interval, lengthening QRS, QRS axis deviation, and T-wave changes), when compared with concomitant assessments of histopathology, have a high predictive value for many forms of heart disease in the rat (Bestetti and Oliveira, 1990). In addition, the QT interval is positively correlated with left ventricular mass in hypertensive rats with reversal of both after antihypertensive therapy, thus demonstrating that the QT interval reflects the phenotypic changes of a mechanically stressed heart (Baillard et al., 2000). This finding indicates that the rat model can inform on repolarization effects as well as arrhythmia risk given that QT interval is altered in human cardiac hypertrophy and ischemia. Also, our laboratory showed that rats exposed to different concentrations of doxorubicin, a known cardiotoxicant, underwent concentration-dependent decreases in heart rate, increased cardiac arrhythmias and ECG abnormalities including QT prolongation, ventricular tachycardia (VT), and ventricular fibrillation that were accompanied by ventilatory deficits, ascites formation, a decrease in core body temperature, weight loss, and ultimately death (Hazari et al., 2009a).

Despite the unique electrophysiological features of the rat heart, the ECG effects of drugs and other agents are comparable with those in other species including humans. There is sufficient correlation between rat and humans in the ECG effects of drugs including tranquilizers (e.g., diazepam and chlorpromazine), antidepressants (e.g., imipramine and amitriptyline), sympathetic agonists (e.g., adrenaline, noradrenaline, isoproterenol, and dobutamine) and vasodilators (e.g., clonidine, dihydralazine, nitroglycerin, and adenosine) (Budden et al., 1981). This qualitative similitude in ECG responsiveness extends to other stressors as well including chemicals, hypothermia, and nutritional deficiencies. Rats do, however, have resistance to specific cardiotoxic agents such as with the cardiac glycoside digitalis; although the reason for this is unclear, the typical ECG effects observed in human toxicity are present at higher doses (Detweiler, 1981). On the other hand, rats respond very similarly to antiarrhythmic drugs. Rats respond to class I (e.g., quinidine and procainamide) and class IV antiarrhythmics (e.g., verapamil and diltiazem) at similar effective dose ranges to that found in other species in models of myocardial ischemia (Curtis et al., 1987), although responses to classes II and III antiarrhythmics are less certain (De Clerck et al., 2002).

**ECG END POINTS IN HUMANS AND RODENTS**

**ECG Morphology (Interval and Contour)**

Chemicals, drugs, and disease all have effects on heart rate–dependent parameters within the human ECG including RR, PR, QRS, and QT intervals (Table 2 lists the various end points derived from the ECG and their clinical utility). As discussed in the introduction, the landmarks within the ECG represent an ordered sequence of electrical events with the PR interval reflecting conduction between the sinus node in the right atrium and the ventricular endocardium, the QRS complex representing total ventricular activation, with the remaining portions of the ECG (QT interval, ST segment and T waves) reflecting ventricular repolarization. Prolonged PR intervals may suggest AV block (block of conduction to ventricles) or proximal conduction system disease (Berne and Levy, 2001). QRS changes (e.g., prolongation or fractionation) may identify individuals with increased arrhythmia risk and have been attributed to bundle branch blocks or ventricular ectopic foci (abnormal pacemaker sites in ventricles) (Berne and Levy, 2001). ST segment depression is characteristic of myocardial (ventricular) ischemia/hypoxia, and ST segment elevation is characteristic of myocardial injury (Berne and Levy, 2001). Ischemic episodes in humans are negative prognostic indicators that point to an increase in the probability of future cardiac events including myocardial infarction (Tzivoni et al., 1988). Abnormal T-wave morphology may indicate underlying pathology. The QT interval, when adjusted for heart rate (corrected QT; QTc), is used to assess abnormalities in ventricular repolarization (because Q waves are often not present in rat and mouse ECGs, the base of the R wave is used in rodent models as a surrogate for the Q). Spatiotemporal heterogeneity of repolarization (as indicated by abnormal T waves and/or QT intervals) may trigger arrhythmias and thus has been used to identify patients at risk for cardiac death (Henneberger et al., 2005).

Unlike in human electrocardiography, there are no specific guidelines to assess rat ECG morphology. Nonetheless, analogous assessments of ECG interval duration, amplitude, and area are usually carried out (Table 2). This approach is justified given the similarity of electrocardiographic responses in humans and rodents.
TABLE 2
ECG Parameters and Their Significance

| ECG interval and contour       | Interval duration, area and amplitude (e.g., QT duration, ST area, and T-wave amplitude) | Changes may suggest ischemia, heterogeneity of repolarization, ion channel dysfunction |
| ECG rhythm                    | Changes in heart rate, irregularity of rhythm                                           | Arrhythmias may be benign or have serious hemodynamic repercussions including no cardiac output |
| HRV                           | Time domain (SDNN, rMSSD, pNN50) and frequency domain (LF, HF, LF/HF, VLF, vHF)         | Changes in the balance of autonomic sympathetic and parasympathetic tone to the heart |

Note. SDNN, standard deviation of the interval between two normal heart beats (NN intervals); rMSSD, root mean square of successive differences in NN intervals; pNN50, percentage of differences between adjacent NN intervals that are > 50 milliseconds; LF, low frequency; HF, high frequency; LF/HF, ratio of LF to HF; VLF, very low frequency; VHF, very high frequency.

Arrhythmia

Xenobiotics also have effects on heart rhythm. Normal cardiac contraction in humans and rodents consists of atrial followed by ventricular contraction and results from an ordered pattern of depolarization (atrial then ventricular depolarization) initiated by spontaneous SA nodal depolarization. An arrhythmia results when this sequence is abnormally slow (bradyarrhythmias) or fast (tachyarrhythmias) or is irregular. Bradyarrhythmias result from enhanced normal suppression or disorders of automaticity of the SA node, and AV heart block (failure of electrical impulse propagation from the atria to ventricles). The failure of SA nodal automaticity is the most common cause of bradyarrhythmia in humans and results in sinus bradycardia or “sick sinus syndrome” (Dresing and Wilkoff, 2001). There are three types of AV block. When the impulse propagates to the ventricles at a slower rate than normal, it is referred to as first-degree heart block (Dresing and Wilkoff, 2001). Second- and third-degree block indicate a greater degree of impairment of conduction with intermittent or no conduction to the ventricle, respectively (Dresing and Wilkoff, 2001). Heart block usually results from disease or drug-induced pathology at the level of the AV node or His bundle-Purkinje system. Bradyarrhythmias are the primary reason for artificial pacemaker implantation (Dresing and Wilkoff, 2001). Tachyarrhythmias, on the other hand, result from three primary mechanisms: enhanced automaticity, re-entry, and triggered activity. Enhanced automaticity takes place when pacemaker tissue other than the SA node (e.g., AV node or Purkinje fibers) acquires faster automaticity or when myocardial cells, which normally lack automaticity, acquire automatic characteristics and can result from a wide variety of pathophysiological states (e.g., ischemia). Re-entry, the most common mechanism of tachyarrhythmia, results when a cardiac impulse re-excites a region that it had already traversed (Berne and Levy, 2001) and is responsible for ventricular fibrillation, the most serious cardiac arrhythmia. Triggered activity is caused by after depolarizations—abnormal early APs that take place during phase 2 or 3 of a normal AP (Berne and Levy, 2001), and also have multiple potential causes (e.g., hyperkalemia, hypercalcemia, and increased catecholamines). Although some arrhythmias are benign with little hemodynamic consequence, more serious arrhythmias can cause the heart to pump inefficiently. One of the key physiological effects of serious arrhythmias is altered blood pressure. Whereas AV nodal tachycardia causes little or no change in blood pressure, ventricular tachycardia (VT) leads to a reduction in mean arterial pressure and pulse pressure. The worst of all arrhythmias, ventricular fibrillation, is associated with very low blood pressure and cardiac output and no palpable pulse (Curtis et al., 1987). Symptoms of arrhythmias depend on the severity of the arrhythmia and may include dyspnea (shortness of breath), syncope (fainting), fatigue, heart failure symptoms, chest pain, and/or cardiac arrest. Frequent causes of arrhythmia include myocardial ischemia (when cardiac cells lack oxygen, they become depolarized, which leads to altered impulse formation and/or altered impulse conduction), changes in cardiac structure that accompany heart failure (e.g., dilated or hypertrophied cardiac chambers, necrosis, fibrosis), drugs, and electrolyte disturbances (primarily K+ and Ca2+) (Berne and Levy 2001; Curtis et al., 1987).

Historically, clinical assessments of human ECG rhythm have been qualitative (i.e., described in terms of occurrences) in large part because of the reliance on brief ECG strips. One common method of ECG rhythm assessment in human medicine involves three steps: (1) analysis of rate, regularity, P waves, PR interval, QRS interval, and QT interval; (2) a search for dropped beats or pauses; and (3) a search for groupings of QRS complexes (Jones, 2008). The Lambeth Conventions (Walker et al., 1988) established guidelines for the study of cardiac arrhythmias in laboratory animals in ischemia, infarction, and reperfusion models (a meeting convened for the purpose of updating these conventions took place in September 2010 in London with a forthcoming
publication). These conventions were drafted to improve comparability between laboratories and provide guidelines for defining, classifying, and quantifying arrhythmias as well as appropriate design, execution, and analysis of experiments. Given the nature of the disease models studied, a focus was placed on ventricular arrhythmias, namely, ventricular premature beats (VPB), bigeminy (two VPBs separated by a normal beat), salvos (ventricular couplets), VT, and ventricular fibrillation. Modern methods have enabled continuous and digitized ECG recording of much longer ECG segments, which has facilitated quantifiable assessments of ECG rhythm including identification and enumeration of various arrhythmias. The methods for reporting arrhythmia data include incidence, number of episodes, and total duration. The frequency distribution characteristics of the latter two parameters (i.e., non-Gaussian) are such that in order for them to be quantifiable, they require transformation. One other common way to report arrhythmia data sets in a quantifiable manner is arrhythmia scores (Gaussian distributed), which are an “arbitrary numerical grading of ventricular arrhythmias in terms of their perceived severity” (Curtis et al., 1987, 413).

Heart Rate and Autonomic Tone

Although HR measurements do not require an ECG, they may be obtained from the RR interval, which enables the determination of abnormal heart rate (i.e., bradycardia or tachycardia). The interval between R peaks in two consecutive QRS complexes represents the duration between two normal heart beats (NN interval) that originate from the sinus node and not those because of ectopic beats (Zareba et al., 2001). Heart rate variability (HRV) is the degree of difference in the interbeat intervals of successive heartbeats and is an indicator of the balance between the sympathetic and parasympathetic arms of the autonomic nervous system (Rowan et al., 2007). Low HRV, reflecting inappropriately increased sympathetic tone (Rowan et al., 2007), is associated with an increased risk of cardiac arrhythmia (Corey et al., 2006) and an increased risk of mortality in people with previous myocardial infarction (Bigger et al., 1993; Fauchier et al., 2004). Time domain measures of variability and frequency domain analysis of the power spectrum of heart rate are the two most common approaches to studying HRV. Time domain parameters are derived from statistical analyses of the variation of the NN interval. These include standard deviation of the NN interval and root mean square of successive differences in NN intervals and generally reflect parasympathetic influence on the heart. Frequency-domain parameters are derived from a spectral analysis of the NN interval (Zareba et al., 2001). High-frequency (HF) and low-frequency (LF) bands, among others, represent parasympathetic and sympathetic control of heart rate, respectively, and are often presented as a ratio (LF/HF) to reflect sympathovagal balance.

The task force of the European Society of Cardiology and the North American Society for Pacing and Electrophysiology (1996a,b) proposed guidelines for HRV assessments in humans that includes standards for HRV measurement, physiological interpretation, and clinical use. No such guidelines exist for laboratory animals in part because of species differences in heart rate and the ECG. One of the key recommendations of the task force was the suggestion that the minimum sampling duration required to derive reliable HRV data be 5-min averages recorded each hour for 24 h. When adjusted for differences in heart rate, 5-min heart rate averages in humans are analogous to approximately 70 s of continuously recorded rat data (Rowan et al., 2007) provide a thorough review of HRV assessments in rodent toxicology studies. Investigators designing rodent toxicology studies should also carefully consider the timing of data collection as rats and mice are nocturnal and HRV parameters in general are very sensitive to movement/stress artifact. Although the utility of HRV assessments as valid prognostic indicators in human heath is widely accepted, because of the limited number of published studies, it is unclear if HRV data in rats are equivalent homologous surrogates of cardiovascular morbidity and mortality. Nevertheless, some studies report links between adverse cardiovascular outcomes and decreased HRV in rats (Fauchier et al., 2006; Huang et al., 2010; Melin et al., 2005; Rhoden et al., 2005; Sugimura et al., 2008), suggesting that these measurements should be explored in toxicology studies. Several rodent-specific commercially available software packages can analyze data obtained from restrained, anesthetized, or freely roaming rodents.

EXPERIMENTAL METHODS IN SMALL LABORATORY RODENTS

In Vivo Monitoring of Surface ECG in Conscious Rats and Mice

In order to understand surface ECG monitoring in rats, a thorough appreciation of the science behind surface ECG monitoring in humans is necessary. The ECG records the sum of the electrical forces generated by all the active individual cells within the heart (that are depolarizing or repolarizing) at the body surface. The shape of the ECG (direction and magnitude of deflections) is determined by how these electrical forces are aligned relative to the reference axes established by the positions of the sensing electrodes on the body (Garibyan and Lilly, 2007). The amplitude or strength of the deflection is determined by the amount of tissue depolarizing or repolarizing at any given time (e.g., the large QRS wave of the ECG results from the depolarization of the large ventricles; atrial depolarization on the other hand results in the smaller P wave). The direction in which a depolarization wave travels relative to the recording electrodes also determines the size of the recorded signal. In human electrocardiography, multiple leads
are used because of the increased accuracy with more leads. The standard 12-lead ECG system used in humans (Fig. 3A) includes the bipolar (lead I, II, and III) and augmented unipolar leads (aVL, AVR, and aVF), which examine electrical forces in the frontal plane of the body, and the chest (or precordial) leads (V₁–V₆), which record electrical forces in a perpendicular plane. The bipolar lead system places leads on the right arm, left arm, and left leg. In this system, one limb is the (+) pole and another electrode provides the (−) reference (Garibyan and Lilly, 2007). The unipolar lead system uses the same three electrode positions as the bipolar leads, but does not rely on a single (−) pole. Instead, one pole is (+) (e.g., aVR) and all the other poles are averaged to generate a (−) reference (Garibyan and Lilly, 2007). The added use of chest leads allows a more three-dimensional assessment of electrical forces, thus allowing recording of more localized changes in cardiac potentials. In the precordial chest lead system, the electrodes are placed around the fourth and fifth intercostal space and their potentials are measured against the potentials of the other three limb leads. Other less commonly used human lead systems include modified chest leads, the right-sided 12-lead ECG, and the 15-lead ECG that are specifically used to detect regional blocks or lesions (Jones, 2008), and surface potential mapping with 64 or more electrodes (Yamada et al., 1975).

The last 30 years has seen a dramatic shift in the nature of ECG monitoring in rodent studies and has thus enabled investigators to adopt ECG methods in rodents that are analogous to human ECG monitoring. Previously, investigators had to rely on physical restraint or anesthesia to enable in vivo assessments using surface electrodes. Restraint, as has been demonstrated in many studies, has profound effects on heart rate, blood pressure, body temperature, endogenous hormone release, and body weight (Driscoll, 1981) and may even lower the threshold for arrhythmia (Schlatter and Zbinden, 1982). Anesthesia has a number of effects on the cardiovascular system as well. For example, general anesthesia alters autonomic tone, whereas inhalation anesthetics, including halothane, enflurane, and methoxyflurane, have been shown to have pro- or anti-arrhythmic effects and inhibit critical Ca²⁺ channels (i.e., halothane and isoflurane) in the heart (Mayo and Jamali, 1999). In addition, anesthetics have also been shown to interact with drugs (e.g., methoxyflurane interacts with verapamil to synergistically prolong the PR interval; Mayo and Jamali, 1999). These contexts may thus potentially confound a natural physiological response to an agent in a toxicology study. These hurdles have been overcome by the advent of implantable radiotelemetry devices that, although dependent on a brief invasive procedure, allow for the acquisition of data from stress-free, conscious, and freely roaming rodents after sufficient recovery time. One of the earliest groups to use implantable telemetry devices to record ECG and heart rate was Schlatter and Zbinden (1982). Implantable telemetry enables continuous monitoring during repeated administration of a toxicant for long periods as well as the assessment of subchronic and chronic effects long after exposure. Such telemetry devices sense electrical signals (ECG

FIG. 3. (A) 12-Lead ECG in humans. Three limb electrodes (placed on the right arm, left arm and left leg) are used to generate six limb leads (leads I, II, III, aVF, aVL, and aVR) and six chest electrodes generate six chest leads (V₁–V₆). (B) Lead II may be achieved in the rat, as depicted in the illustration, by placement of the negative electrode near the right shoulder and the positive electrode to the left of the xyphoid space, analogous to the human right arm (RA)–negative electrode and left leg (LL)–positive electrode configuration.
and HR) and then transmit them to receivers positioned near the implanted animal; data is collected using acquisition software (Kramer and Kinter, 2003; Stohr, 1988). Data output from the acquisition system can then be analyzed using analysis software, which has replaced arduous and less accurate assessments of ECG printouts. There are several commercially available computer programs that enable automated analysis of HR, HRV ECG, blood pressure, body temperature, and activity among others. The affordability and reliability of radiotelemetry measurements have improved such that its use in small rodents has dramatically increased over the past two decades. The implantation procedure most often requires abdominal insertion of the telemetry device body with positive and negative electrodes placed subcutaneously (Kramer and Kinter, 2003; McCauley and Wehrens. 2010; Terasaki et al., 1990). A 2-week recovery time is usually needed and recommended before commencement of the study. According to several studies, physiological parameters at baseline are lower in animals implanted with a radio-telemetry transmitter than those obtained with traditional techniques using restraint (Kramer and Kinter, 2003). Although the rat’s larger size facilitates telemeter (ECG transmitter) implantation procedures, mouse telemetry is becoming more common because of the greater availability of mouse-specific reagents and transgenic models compared with rats.

There are no guidelines available for lead configurations in toxicology studies. The Lambeth Conventions (Walker et al., 1988), which established guidelines for the study of arrhythmias in animal models of ischemia, infarction, and reperfusion, stipulate that a clear P wave during sinus rhythm (i.e., rhythm initiated by the sinus node) is required. Despite anatomical differences with humans and the absence of guidelines, analogous lead systems have been used in rats using implantable telemetry devices. The subsequent discussion of the various lead systems in rats and mice will largely be limited to studies performed in the last 15 years because of improved methods and the reduced reliance on restraint or anesthesia. All three leads of the Nehb-Sporri system have been used simultaneously in anesthetized rats (Driscoll, 1981). Six-lead systems analogous to human ECG leads I, II, III, aVR, aVL, and aVF have been used in restrained rats (e.g., Lake et al., 1987) and anesthetized mice (e.g., Berul et al., 1996; Oikonomidis et al., 2010; Sampson et al., 2008). Twelve-lead systems that include the six limb leads as well as the six chest leads have been used in anesthetized rats (e.g., Kmeova and Klimas, 2010). As is apparent, the utilization of multiple lead systems, particularly 6- or 12-lead systems in conscious unrestrained rats would be technically more challenging and to our knowledge has not been done.

Although implantable telemetry provides tremendous utility in the acquisition of ECG and HR data from conscious and unrestrained rats, there is still a reliance on an invasive surgical procedure for placement of the telemeter. Recent innovations in implantable telemetry technology have enabled less invasive procedures. One totally noninvasive method requires the mouse/rat to sit on a platform embedded with electrodes (Xing et al., 2009; Mouse Specifics). ECG data obtained with this method compares favorably with data obtained from invasive telemetry methodologies (Hoyt et al., 2007). An exciting recent innovation by Pereira-Junior et al., (2010) bypasses the need for any invasive procedure as well. They have devised a system analogous to jacket methodologies in dog studies whereby a rat is fitted with a jacket across its thoracic cavity containing electrodes that allows ECG and HR to be recorded non-invasively in conscious rats. Although this new method requires restraint, future improvements in this procedure allowing measurements in free-roaming rats would significantly simplify ECG studies in rodents.

In Vivo Monitoring with Cardiac Pacing

In some instances, surface ECG recordings, which are derived from placement of electrodes on the skin, are coupled
with placement of electrodes on the surface of the heart to enable myocardial pacing. Berul et al., (1996) performed one of the first studies that combined open heart pacing with simultaneous six-lead surface ECG recordings in mice. This methodology enables the assessment of the impact of direct myocardial stimuli on the surface ECG. Other non-ECG parameters may be measured including sinus node recovery times, effective refractory periods, and monophasic action potentials (MAP). MAP represents the extracellular potential difference between an active and inactive (depolarized by localized pressure) site on the heart (Kondo et al., 2004). MAPs may enable the localization of ischemic areas within the heart (Franz et al., 1984) and help elucidate mechanisms of arrhythmia. Other investigators have used programmed electrical stimulation to assess ventricular refractoriness and arrhythmia induction (Gutstein et al., 2003). Although these procedures rely on open heart surgery and anesthesia, they enable the acquisition of information that would otherwise be impossible to obtain because of ethical and technical considerations.

Ischemia-Reperfusion

One very common means used to elicit experimental myocardial injury is through sequential ischemia and reperfusion (IR). This procedure generally involves temporary occlusion of one of the coronary arteries such as the left anterior descending (for as little as 5 min and up to 120 min) followed by release of the occlusion and reperfusion (Redel et al., 2008). The location and duration of the occlusion determines the size and severity of the lesion. This procedure is designed to mimic the acute ischemia that takes place in reversible myocardial ischemia, myocardial infarction, coronary vasospasm, or thrombosis (Janse et al., 1998). Coronary ligation (ischemia) causes myocardial hypoxia and cytokine release, which combine to trigger intracellular sodium and calcium overload, the mitochondrial membrane permeability transition, osmotic stress, oxidative stress, neutrophil recruitment, and influx to the tissue during reperfusion, which may exacerbate the lesion further (Janse et al., 1998). To monitor ECG during and after the IR procedure, surface electrodes are attached in one or more of the limb-lead configurations (Dhalla et al., 2009; Sakamoto et al., 1999). The nature of this terminal procedure as such necessitates anesthesia. Both rat and mouse models have been used extensively to study the etiology of cardiac arrhythmias resulting from ischemia-reperfusion injury (Wehrens et al., 2000). Some of the common IR-related abnormal changes in the ECG include R-wave enlargement, development of Q-waves, and ST-segment elevation. This model has proven useful in the assessment of the efficacy of antiarrhythmic and anti-ischemic drugs (Burger et al., 2009; Dhalla et al., 2009; Gardiwal et al., 2009; Gandhi et al., 2009; Wajima et al., 2004; Wehrens et al., 2000). IR has also been used as a challenge procedure to determine if toxicant treatment can modify responses to myocardial injury. Such models have been used in the context of allergen treatment (Hazarika et al., 2004; Hazarika et al., 2007) and air pollution exposure (Cascio et al., 2007; Cozzi et al., 2006, 2007).

Ex Vivo Isolated Perfused Heart

The heart is regulated in part by external neurohumoral and hemodynamic factors that help modulate its activity. The isolated perfused heart methodology (first characterized by Oscar Langendorff in 1895 [Anderson et al., 1990]) allows the investigator to characterize the cardiotoxicity of a drug or chemical in the absence of these regulatory influences while maintaining the structural (i.e., coronary vasculature and collagen scaffolding) and functional integrity of the heart (Anderson et al., 1990; Opie and Lubbe, 1979). An additional advantage is the ability to control experimental conditions including drug/toxicant concentration, coronary perfusion pressure, and perfusate composition. The modern, modified, Langendorff preparation is currently used to derive functional (left ventricular pressure and rate of pressure change [dp/dt]), metabolic (including rate of oxygen consumption and fatty acid and glucose metabolism in the perfusate), and electrocardiographic data (Anderson et al., 1990). There are several ways to obtain ECG data using the Langendorff model. Surface electrodes (bipolar) may be attached directly to the myocardium to obtain atrial and/or ventricular ECGs. Choisy et al., (2007) placed electrodes on the epicardial surface of the heart near the apex of the left ventricle and on the cannula to the left atrium. The Langendorff procedure has been recently combined with novel methods including mapping experiments to increase understanding of cardiac function. For example, mapping experiments that make use of hundreds of microelectrodes have been used to monitor functional/hemodynamic changes including left ventricular pressure and coronary flow (Ritter et al., 2010) and voltage-sensitive fluorescent dyes have been used to study electrical activity (Sill et al., 2009). The Langendorff is often coupled with imaging technologies such as intracellular ion-sensitive or metabolite-sensitive fluorophores. For example, the distribution of membrane proteins and functional cell-to-cell coupling in cultured cardiac cells may be evaluated through fluorescence recovery after photobleaching (Muller-Borer et al., 2004).

APPLICATIONS IN TOXICOLOGY

Prevalence of Rat/Mouse ECG Studies

Although electrocardiography assessments in mice are increasing, rat electrocardiography studies are still more common. A recent PubMed literature search revealed that the number of published mouse electrocardiography studies since 1990 was...
873 (key words: mice, electrocardiogram); for rats, 1817 (key words: rat, electrocardiogram) (a similar search in dogs yielded 862 studies; PubMed, 2010). These reports largely describe acute and chronic pharmacology and toxicology studies that include the monitoring of the cardiotoxic potential of various agents/stressors and studies designed to elucidate mechanisms of action. Many of these studies depict previously undescribed electrocardiographic effects of agents as well as insight into possible mechanisms of action of known cardiotoxicants highlighting the potential utility of small rodent electrocardiography.

Xenobiotics

A full appreciation of the utility of electrocardiography in toxicology comes from an examination of the xenobiotics known to elicit cardiotoxicity. They span a broad and diverse range that includes ethanol, chloroform, heavy metals, marijuana, endotoxin, antineoplastics (e.g., doxorubicin), antibiotics, antihistamines, antidepressants, the various classes of molecules used to treat hypercholesterolemia and diabetes (Haschek and Rousseaux, 1998), and many others. This relatively unpredictable and seemingly random grouping of agents has necessitated assessments of electrocardiographic responses in many applied toxicological contexts. Multiple rat and mouse studies with many of these agents have produced electrocardiographic findings largely in concordance with available human data. For example, QT interval is prolonged with doxorubicin treatment in humans and rats (Ferrari et al., 1996; Hazari et al., 2001). Additionally, endotoxin decreases HRV in both humans and mice (Fairchild et al., 2009).

Environmental Stressors

Evidence is growing that implicates the influence of environmental factors on the initiation and progression of cardiovascular diseases. Among them are diet, hypoxia, and exposure to air pollutants (O’Toole et al., 2008). Nutritional deficiencies and diet (obesity) have clear cardiovascular impacts in humans (O’Toole et al., 2008), which manifest in the ECG and have been demonstrated in small rodent models (Detweiler, 1981). Hypoxia has adverse cardiovascular impacts that are readily demonstrable in the ECG including right axis deviation, right bundle branch block, and changes to P and T wave amplitudes (Windsor et al., 2010); similar findings have been demonstrated in small rodents (Hemdahl et al., 2005). The ischemic effects of environmental tobacco smoke have also been demonstrated in both humans and rat models (Glantz and Parmley, 2001). Ambient air pollution poses significant environmental risk as well. The most culpable ambient air pollutants include particulate matter and associated copollutants including nitrogen oxides, sulfur oxides, and carbon monoxide. Small upward fluctuations in each of these agents have been associated with increased cardiovascular morbidity and mortality (Bhaskaran et al., 2009; Dockery, 2001). Some of the most prominent adverse cardiovascular effects include changes in heart rate, HRV, and the induction of cardiac arrhythmias in both humans (Brook et al., 2003) and rodent models (Farraj et al., 2009, 2010; Hazari et al., 2009a). Rodent electrocardiography may be at the forefront in the effort to increase our understanding of the health impacts of environmental stressors including emerging issues like climate change and environmental nanoparticle exposure.

Safety Assessment

In the pharmaceutical setting, compounds of interest are studied for their therapeutic potential in humans and veterinary animals and are thus guided in part by regulatory mandates. Of all assessments of cardiovascular toxicity in this context, the ECG is “the most sensitive early indicator of cardiac toxicity or malfunction” (Gad, 2001, 37) and, accordingly, ECG assessments are conducted within two main spheres that are not mutually exclusive. One is the assessment of cardiotoxicity as part of a larger effort to assess overt whole system toxicity. In this setting, the primary screen for cardiotoxicity consists of selected parameters (usually includes ECG) that are incorporated into standard systemic toxicity studies (Gad, 2001). The second sphere is “safety pharmacology,” which examines pharmacological effects in specific target organs using functional assays (Gad, 2001). The purpose of profiling of pharmacological effects is to search for desirable therapeutic effects of a new compound as well as potential side effects at therapeutic dose ranges (Gad, 2001). According to a survey published in 2001 of methods used by the pharmaceutical industry (a total of 54 international companies of varying sizes) to collect nonclinical cardiac electrophysiology data for “new active substances,” 26% of all laboratories collected ECG data from rats, with most using dogs (Hammond et al., 2001). Whereas none of these laboratories used rats exclusively, these findings demonstrate that small rodent models contribute to the overall evaluation of electrocardiographic effects of compounds in safety assessment. The primary reason the dog continues to be the species of choice to assess ECG effects in the pharmaceutical industry revolves around the potential effects of drugs on the QT interval (Gralinski, 2003). QT interval prolongation is of particular concern because of its association with serious cardiac arrhythmias including torsade de pointes that can lead to potentially lethal ventricular fibrillation (Gralinski, 2003). These effects are believed to be because of the targeting of the delayed rectifying potassium currents that govern repolarization (e.g., IKr and IKs) by these drugs (Gralinski, 2003). The ion channels that predominately carry these currents are the same in humans and dogs (the dog ECG also exhibits human-like respiratory sinus arrhythmia; Shykoff et al., 1991; Detweiler, 1997). Rats are believed to be less susceptible to these effects because of their dominant transient outward current (IKo), which is believed to override...
any effect on IKr (Gralinski, 2003). Whereas the rat ECG’s usefulness is in question with respect to identifying agents that affect the QT interval, multiple studies have shown that pathophysiological changes associated with toxicant exposure or disease overall manifest similarly in the ECG of rodents and humans (Bestetti and Oliveira, 1990; Detweiler, 1981). The suitability of small rodent electrocardiography as a stand-alone methodology or as a replacement for dog models as well as the appropriate stage of drug development rodent models that should be used in drug development requires further investigation and is beyond the scope of this review. Future studies that compare the effects of agents known to impact different regions of the ECG in both dogs and rats/mice will add to the understanding of the translational impact of rodent models as well as their utility in safety assessment.

Study Design

The undertaking of electrocardiographic assessments in toxicology must come with an understanding that the goals of ECG assessments in toxicology differ from their use in clinical electrocardiography. Detweiler (1983) outlined several reasons why the ECG should be monitored in toxicology including: 1) changes in electrophysiology are a principle manifestation of cardiotoxicity, 2) death from cardiovascular injury/disease is often preceded by changes in the electrical activity of the heart, which can be monitored noninvasively, 3) many toxicants elicit ECG lesions (i.e., ECG effects) without causing any discernible myocardial lesions, 4) animals having ECG abnormalities can be identified and removed before the commencement of exposure (i.e., prior to treatment or exposure), 5) and continuous ECG monitoring may help determine the onset of toxic effects in agents that produce cardiac lesions. Most of the rat and mouse studies predating 1990 used restraint procedures or terminal anesthetized preparations that were limited to ECG assessments after exposure. Such anesthetized preparations are currently used by many investigators because they are ideal for limiting signal artifact (from skeletal muscle electrical activity or movement) and allow for techniques such as intracardiac pacing or challenge with arrhythmogenic agents that would otherwise be impossible in conscious animals. As aforementioned, modern technology has shepherded in several advances in ECG assessments including continuous monitoring of the ECG in unanesthetized unrestrained animals and the digitization of ECG data such that ECG parameters are readily quantifiable (i.e., enable calculation of means and standard errors). Continuous monitoring has the added advantage of allowing a period of acclimatization thus enabling stable and reproducible baseline pre-exposure measurements. We and others (Nadziejko et al., 2002) have shown that baseline ECG, heart rate, HRV, and arrhythmia data often vary among animals, particularly in strains with pre-existing disease, and thus postexposure values alone have limited utility. This point is exemplified in Figure 4, which shows PR, QRS, ST, and QT interval data obtained from our recent study in hypertensive and normal rats before, during, and after subchronic treatment with the anthracycline doxorubicin (the ST interval depicts the length in time between the S of the QRS and the peak of the T wave in all species, whereas the ST segment depicts the isoelectric portion of the ST interval in species that possess this isoelectric portion within their ECG; Hazari et al., 2009b). The baseline differences between groups of the same strain strongly suggest that postexposure ECG-related data should be normalized to subject-matched baseline data. This normalization should be time matched as well, given the impact of the diurnal cycle/circadian rhythm on these values. Figure 5 shows examples of normalized ST amplitude and HRV (LF/HF) data from our recent study in rats exposed to particulate air pollutants (Farraj et al., 2011). Manifestation of cardiotoxic effects after exposure to a xenobiotic may be immediate or take months to appear and thus monitoring duration is a critical consideration in study design. For continuous monitoring studies, ECG/HR sampling intervals must be designated before the commencement of monitoring. For derivation of HRV data in particular, sampling intervals are often selected to reflect the standard espoused by the European Society of Cardiology and the North American Society for Pacing and Electrophysiology (1996a,b) for human HR data extrapolated to mouse or rat heart rate.

ECG Monitoring during Challenge/Stress Tests

Whereas ECG monitoring during and after exposure is very useful for detecting overt cardiotoxic effects, often effects are more subtle and only manifest when a stressor is encountered. In some instances, xenobiotics may condition potentially susceptible individuals lowering their response thresholds to otherwise typical triggers that are compensated within normal homeostatic bounds. Clinical researchers use developed “challenge” testing to measure the risk of such conditioning or altered threshold—e.g., nonspecific airway challenge in asthmatics. This concept has led to methods that focus on assessing human physiological (functional) responses to stressors that may trigger dysfunction and in the extreme may even cause sudden death. In humans, treadmill (exercise) testing with ECG monitoring is used to “stress” the cardiovascular system in order to assess the risk of future events. The objective is to stress patients with mild to moderate exercise, which increases heart rate, contractility, and cardiac workload. Patients are then monitored for ECG abnormalities that may indicate myocardial ischemia (attendant to coronary artery disease), or a predisposition to a catecholamine-dependent arrhythmia. The ST segment of the ECG is a particularly useful indicator during exercise (Mills et al., 2007; Pekkanen et al., 2002) because oxygen demand is elevated during exercise thus increasing the likelihood of ischemic episodes, which in turn may be reflected in ST changes in the ECG (Scheidt, 1986). This type of testing has also been adapted into animal models of cardiovascular disease in rats (Mautz, 2003).
Although rodent treadmills are available and widely used, training and consistency in performance can often be a challenge for researchers. Thus, just as in human beings who are unable to perform exercises because of physical limitations, dobutamine, which is a direct-acting sympathomimetic drug that primarily stimulates beta-1-adrenergic receptors on the heart, resulting in increased heart rate and contractility, can be administered intravenously as a cardiac stress test in place of exercise to assess cardiac electrical dysfunction and abnormalities in the ECG. Functional assessments on the feasibility of dobutamine stress echocardiography have shown that it can easily be performed in laboratory rats, which respond with an increase in heart rate and ejection fraction, and a decrease in left ventricle dimensions (Plante et al., 2005). By removing the echocardiography component, we have been able to measure the physiological responses (heart rate, ECG, PR interval, QRS duration, ST segment duration, QTc) in laboratory rats and compare them with vehicle-treated rats.

FIG. 4. Doxorubicin (DOX)-exposed normal (WKY) and hypertensive (SH) rats. Low (1.25 mg/kg), medium (med; 2.5 mg/kg), and high (5.0 mg/kg) DOX caused significant increases in the PR interval of WKY rats (A, top row), whereas there was no change in SH rats (B, top row), which had longer pre-existing PR intervals than WKY rats. High DOX caused a significant increase in QRS duration in WKY rats when compared with baseline and vehicle-treated WKY rats (A, second row), but no effect in SH rats (B, second row). High DOX caused a significant increase in ST interval duration when compared with vehicle in WKY rats (A, third row), but no effect in SH rats (B, third row). High DOX caused a significant increase in QTc when compared with vehicle in WKY rats (A, second row), but had no effect in SH rats. *Significantly different from vehicle, \( p < 0.05 \) (Modified from Hazari et al., 2009b). Note the differences among the groups in the baseline ECG values, particularly in SH rats.

**Dobutamine “Stress” Test**

Although rodent treadmills are available and widely used, training and consistency in performance can often be a challenge for researchers. Thus, just as in human beings who are unable to perform exercises because of physical limitations, dobutamine, which is a direct-acting sympathomimetic drug that primarily stimulates beta-1-adrenergic receptors on the heart, resulting in increased heart rate and contractility,
Arrhythmogenesis) in conscious unrestrained rats surgically implanted with radiotelemeters and a chronic intravenous catheter. Our laboratory has demonstrated that a single exposure to a toxic air pollutant (diesel exhaust or acrolein gas) significantly increases the occurrence of cardiac arrhythmias in rats during the dobutamine stress test 24 h after pollutant exposure (Hazari, Haykal-Coates, Lamb, Winsett, Krantz, King, Costa, and Farraj, in preparation). Furthermore, ST depression and QTc prolongation are only observed in rats with underlying cardiovascular disease (e.g., hypertension) during dobutamine challenge, suggesting parallels between susceptible human populations and rats. These preliminary results indicate that the stress of repeated sympathetic stimulation, which mimics exercise conditions, increases the risk of an adverse cardiac event after exposure to air pollution. Thus, this methodology can be used to elucidate the underlying mechanisms of adverse cardiac health effects, which is not always easy, or even possible, in human studies.

Aconitine “Stress” Test

A slightly less intuitive method of cardiac challenge employs aconitine, which is a cardiotoxic alkaloid derived from the plant Aconitum (wolfsbane) and widely used to produce ventricular arrhythmias in laboratory animals. Aconitine targets voltage-gated sodium channels in various excitable tissues, including the myocardium, suppressing their inactivation and thereby interfering with repolarization of the cardiomyocyte membrane in preparation for the next beat. This drug has been used to produce experimental arrhythmia, often as a positive control in drug testing paradigms assessing potential arrhythmogenic properties (e.g., doxorubicin—Dragojevic-Simic et al., 2004) and the efficacy of antiarrhythmic drugs (Bartosova et al., 2007; Lu and De Clerck 1993). However, the toxicity of aconitine precludes its use in humans as well as conscious rodents. Continuous slow infusion of aconitine in anesthetized rodents produces VPB, eventually worsening to VT and then ventricular fibrillation thereafter. We have previously shown that hypertensive rats (with external telemeter attached to the skin in a lead II configuration to monitor ECG) are more sensitive to the arrhythmogenic effects of aconitine when compared with healthy rats, suggesting that host factors (e.g., cardiovascular disease) increase vulnerability to triggered arrhythmias (Fig. 6; Hazari et al., 2009a). Moreover, the same study demonstrated that a single exposure to particulate or gaseous air pollution further increases the sensitivity of rats to aconitine-induced arrhythmia. This methodology can be employed to study mechanisms of adverse cardiovascular health effects, particularly by incorporating pharmacological interventions (e.g., antihypertensive agents) and use of genetically modified rodents.

Interpretation

Once ECG data are obtained, the data must be interpreted, including statements on the potential/likely cause(s) of toxicity. Toxicants may (1) affect ion homeostasis (e.g., the inhibition of Na\(^+\),K\(^+\)-ATPase by cardiac glycosides, Ramos et al., 2001, and the antagonism of endogenous Ca\(^{2+}\) by the heavy metal cobalt, Haschek and Rousseaux, 1998), (2) alter coronary blood flow (e.g., vasoconstriction induced by the catecholamine epinephrine; Ramos et al., 2001), (3) trigger oxidative stress (e.g., alcohol and doxorubicin increase the production of reactive oxygen species, which react with membrane phospholipids and inhibit the function of critical enzymes; Ramos et al., 2001), (4) cause organelle dysfunction (e.g., the immunosuppressant FK506 alters sarcoplasmic reticulum function and cyanide disrupts mitochondrial function; Ramos et al., 2001), (5) initiate apoptosis (e.g., cocaine causes the release of apoptotic signals that mediate programmed cell death; Ramos
FIG. 6. Exposure to varying concentrations of residual oil fly ash-like particulate matter or the irritant gas acrolein increases the risk of developing cardiac arrhythmias. Constant infusion of aconitine (2 μg/min) triggers VPB followed by VT, ventricular fibrillation (VF), and progression to cardiac arrest (CA) in air-exposed spontaneously hypertensive rats. The cumulative dose of aconitine necessary to trigger VPB, VT, VF, and CA in rats exposed to increased concentrations of particulate matter (s-ROFA: residual oil fly ash-like synthetic particulate matter) or acrolein was significantly lower than air-exposed controls. *Significantly different from air-exposed control; p < 0.05 (Modified from Hazari et al., 2009a).

et al., 2001), (6) cause structural changes (e.g., isoproterenol causes cardiac hypertrophy; Carll et al., 2010), (7) affect extracardiac structures or organ function that change cardiac hemodynamics (e.g., monocrotaline causes right ventricular hypertrophy via the induction of pulmonary hypertension; Ghodsi and Will, 1981), and/or (8) influence the central hemodynamics (e.g., monocrotaline causes right ventricular extracardiac structures or organ function that change cardiac hemodynamics (e.g., monocrotaline causes right ventricular extracardiac structures or organ function that change cardiac hemodynamics (e.g., monocrotaline causes right ventricular expenditures). When combined with novel and sophisticated electrophysiological techniques that can only be done in animal models or in vitro, ECG assessments may be used to derive and tremendous mechanistic insight. These developments, along with the demonstrated validity and utility of the rodent ECG and the growing list of known human cardiotoxicants, suggest that ECG assessment in small rodents is an invaluable tool that will serve to advance the field of toxicology.

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