



Original article

Evaluation of cardiovascular and ECG parameters in the normal, freely moving Göttingen Minipig

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ABSTRACT

Introduction: The objective of this study was to evaluate the normal cardiovascular and ECG parameters in freely moving minipigs and to use these data as the basis of pharmacological drug safety evaluation. **Methods:** 7 Göttingen Minipigs were equipped with radiotelemetry transmitters (ITS). Aortic pressure (AP), left ventricular pressure (LVP), lead II of the ECG and body temperature were continuously monitored. Noto cord HEM 4.2 software was used for data acquisition. Power calculations for the various parameters were done to assess appropriate sample sizes. **Results:** We obtained excellent signal quality and found stable hemodynamic parameters with a low intrinsic heart rate in the Göttingen Minipig. After oral dosing of vehicle, the hemodynamic parameters returned quickly to baseline values indicating that the procedure was well tolerated. The heart rate dependency of the QT interval had to be corrected individually. A sufficient power could be achieved with a sample size of 4 due to the low variability of the parameters measured. **Discussion:** These are, to our knowledge, the first data documenting the course of systemic arterial and ventricular hemodynamic parameters in the freely moving Göttingen Minipig over 24 h. As such, they may serve as a basis for future studies in which drug effects are studied in these animals.

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1. Introduction

Pigs are being used increasingly in biomedical research, particularly in pharmacological and toxicological testing. Preclinical testing for tolerability and safety is required in both rodents (usually the rat) and in a second, non-rodent species (Guth, Germeyer, Kolb, & Markert, 2004; Pugsley & Curtis, 2006). The dog has been the non-rodent species most commonly used for toxicity studies (Lehmann, 1998). Nevertheless, there are cases in which the dog is not suitable. This can be due to dog-specific drug malabsorption, metabolism or any other atypical response that can impact on drug safety. Furthermore, a pharmacodynamic response to a given agent is needed to qualify a species for toxicological testing. If a given pharmacodynamic effect cannot be demonstrated in the dog, alternative species must be considered. The primate has been widely used when the dog has been shown to be inappropriate, but animal availability and ethical considerations limit the use of primates. Thus, further options for non-rodent safety pharmacology and toxicity testing are needed.

The domestic pig, due to its large size as an adult (can exceed 100 kg bodyweight), has not been a commonly used animal for research purposes. In 1949, first attempts were made to develop smaller pigs ("miniature pigs") at the University of Minnesota (Bustad & McClellan, 1968). Today, several different breeds of minipigs are available that are suitable for laboratory use since they reach weights of only 20–35 kg. The Göttingen Minipig is one such breed, having a consistent phenotype, exhibiting a friendly behaviour suitable for handling in the laboratory environment (Lehmann, 1998).

In the meantime, use of pigs for biomedical research has demonstrated that they offer potential advantages, when certain comparisons to man are desirable. For instance, the minipig has a similar heart-to-body weight ratio and a coronary artery distribution similar to humans. Moreover, cardiac anatomy, metabolism and electrophysiology is comparable to man (Bollen & Ellegaard, 1997; Crick et al., 1998; Glodek & Oldigs, 1981; Henke, Brill, & Feußner, 2005; Hughes, 1986; Khan, 1984; Leucht, Gregor & Stier, 1982; Zambraski & Fuchs, 1980). First analyses show that the major myocardial ion currents responsible for the human myocardial action potential are also present in the Göttingen Minipig (Poster Presentation on SPS Meeting 07). At the same time, use of the dog as an experimental animal has drawn criticism due to its role as a companion animal (Bollen & Ellegaard, 1997; Hughes, 1986; Khan, 1984; Rollin, 1986). In contrast, the pig is still viewed primarily as a farm animal with fewer emotional and ethical implications.

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As a consequence, minipigs are being more extensively used not only for cardiovascular biomedical research (Bustad & McClellan, 1968; Kuwahara et al., 1999), but as an alternative non-rodent species for toxicological studies (Khan, 1984; Lehmann, 1998). In spite of this trend, there are few cardiovascular and electrocardiographic reference data available from minipigs in the literature. Most of the published hemodynamic data have been collected with invasive measurement techniques (Beglinger, Becker, Eggenberger, & Lombard, 1975). ECG data from normal minipigs is available from animals restrained in a sling with external limb leads (Dukes & Szabuniewicz, 1969, Eckenfels & Schuler, 1988; Larks, Wescott, & Larks, 1971; Nahas, Baneux, & Detweiler 2002, Suzuki et al., 1998). To our knowledge there are no published data for left ventricular pressure from normal, freely moving minipigs. LVP measurements have been done only in anesthetized (Benharkate, Zanini, & Blanc et al., 1993; Diederer & Kadatz 1981; Lal, Bhattacharya, & Dadkar, 1981; Smith, Spinale, & Swindle, 1990; Vainio, Bloor, & Kim, 1992) or in awake, sling-restrained animals (Vainio Bloor, & Kim, 1992). LVP measurements are particularly useful in that the derivative of LVP, i.e. LV dp/dt , is a well-established parameter for the assessment of cardiac contractility (Klumpp et al., 2006, Markert et al., 2004, Markert et al., 2007). Thus, one goal of the present work was to establish baseline hemodynamic data, including left ventricular hemodynamics and contractility, in freely moving, conscious minipigs.

We recently established a telemetric animal model using Göttingen Minipigs for safety pharmacology assessments. A fully implantable telemetric system was used with pressure transducers in the left ventricle and the descending thoracic aorta connected to a transmitter unit and with integrated ECG leads. This is to our knowledge the first application of this system in the minipig. In the present study, we evaluated hemodynamic (heart rate, aortic pressure, left ventricular pressure, LV dp/dt max) and electrocardiographic (PR, QRS, QT, RR) parameters, as well as body temperature, in 7 normal, freely moving Göttingen Minipigs over 24 h. Diurnal effects were analyzed and we also assessed the dependency of the QT interval duration on heart rate using various correction models.

2. Methods

2.1. Telemetry system

The telemetry system used to measure cardiovascular parameters is manufactured by Konigsberg Instruments, Inc. (Pasadena, CA) and marketed by RMISS (Delaware). It consists of 5 major components: a) an implantable unit; b) a receiver (antenna) located in the animals cage together with an amplifier; c) ambient pressure monitor to measure atmospheric pressure; d) a PC-based “base station” to receive and process the amplified signals; and e) a PC-based data acquisition system (NOTOCORD Hem 4.2) to process signals.

The implantable unit (“T27” total implant) consists of: (1) two high fidelity pressure transducers (4.0 mm diameter), (2) ECG cable; (3) micro-power battery-operated electronics that process and digitize the information from the pressure transducers and the ECG lead, (4) a radio-frequency transmitter that sends the signals to the telemetry receiver, and (5) a battery. A small cable projecting from the transmitter contains a switch that allows the device to be turned on and off to prolong battery life.

2.2. Animals

Treatment of the animals followed the German Law on the Protection of Animals and was performed with permission from the Baden-Württemberg Animal Welfare Committee.

For this study we used 7 Göttingen Minipigs obtained from Ellegaard Göttingen Minipigs Aps, Dalmose, Denmark. The Göttingen Minipig is a cross between the Vietnamese potbelly pig, Minnesota

Minipig and the German landrace pig. It is now a genetically outbred minipig available for research. Our animals were of both sexes (4 males, 3 females), at least one year of age and weighed 22–32 kg. The minipigs were housed in our AAALAC-accredited facility individually or in pairs in separated pens. Room temperature (21 ± 2 °C) and humidity ($60 \pm 15\%$) was controlled with a ventilation turnover of 12/h. They had access to water ad libitum and were fed ~300 g per animal (~25 kg) of a solid standard minipig diet (SDS SMP(E) from Special Diets Services, Witham, Essex, U.K.; supplier SDS Deutschland, Altrip) adapted to body weight once daily. The minipig pens were enriched with wood shavings and sleeping boxes with straw, metal chains, rubber nipples to bite and balls to play with. Furthermore, they received a daily training from our animal staff and were weighed at least twice a week. To monitor the health status of the minipigs, blood is collected every 3 months to determine blood count and clinical parameters, including electrolytes and kidney parameters. Before surgery each animal underwent a routine veterinary health inspection and a short check (about 15 min) of the external ECG taken while resting in a sling.

The minipigs were already well-trained in the telemetry lab, a process beginning at least 2 months before surgery (e.g. p.o. application, general handling, etc.). Room temperature and humidity was controlled (22 ± 2 °C; $60 \pm 15\%$ humidity) with a ventilation turnover of 13/h. We maintained a 12 h light (365 lx) and dark (3 lx, “moonlight”) cycle. Background “noise” was supplied using a radio during the lighted period.

2.3. One-time surgical implantation

The transducers of the T27 implant were calibrated and the unit was sterilized using a low pressure ethylene oxide process prior to implantation. All procedures were conducted under strict aseptic conditions.

For perioperative analgesia the minipigs were given Metacam (meloxicam, 0.4 mg/kg). Additionally, a second analgesic component was given: 2–4 Durogesic smat plasters (fentanyl, 25 µg/h) were attached behind the ear the day before surgery after shaving and degreasing the skin. During surgery a supplemental fentanyl infusion (0.005 mg/kg/h) was given. For prophylactic antibiotic treatment, Duphamox LA (amoxicillin, 15 mg/kg) was given beginning the day before surgery.

Animals were sedated with a combination of Ketavet (ketamine hydrochlorid, 15 mg/kg i.m.) and Dormicum (midazolam, 0.35 mg/kg i.m.). Propofol Lipuro 1% (propofol, ~2 mg/kg) was then administered i.v. to allow endotracheal intubation. The dose was dependent on effect and administration was performed very slowly to prevent depression of the respiratory system. After preparing the animals skin for surgery under aseptic conditions, anesthesia was maintained throughout the remaining procedure with isoflurane (1–3%) inhalation anaesthesia with mechanical ventilation (66% O₂) at a ventilation rate of 14/min.

The minipigs were placed in a lateral recumbency with the left side facing the surgeon. An incision was made near the fifth or the sixth rib. The preparation through the connective tissue and the muscles was continued until the rib and the intercostal muscles were reached. As next step a small pocket was opened in the abdominal wall for implantation of the transmitter, battery housing and induction switch coil. The cables with both pressure transducers and the ECG leads extending from the transmitter were guided subcutaneously to the lateral incision. The antenna was guided subcutaneously from the transmitter location ventral towards the linea alba and further parallel to the mamma complexes in a cranial direction.

The initial ventral incisions required for battery and transmitter placement were closed. A left thoracotomy was then performed after removing the fifth or sixth rib to provide access to the left ventricle apex and the thoracic aorta. After insertion of the left ventricular Konigsberg transducer and fixing it in place with a purse string suture, the aortic pressure transducer, which also served as one electrode of the ECG, was implanted just below the aortic arch. The other electrode

was placed toward the sternum under ECG-signal control to ensure a good (with lead II configuration) signal quality. After the implantation, the lung was inflated, the intercostal muscles were sutured closed and the pneumothorax evacuated. Chest incisions were closed.

The gas anaesthesia was then turned off and the minipigs were allowed to wake up. Antibiotics were administered for 10 days post-operatively. To ensure postoperative analgesia, the transdermal fentanyl plaster was kept in place for 4 days and meloxicam administration was continued for 10 days.

The animals were given at least 21 days for a full recovery prior to the initiation of experiments.

2.4. Experimental design

Forty-four experiments were carried out with the 7 instrumented minipigs. The experimental design was that used with our safety pharmacology studies (Fig. 1). The animals were put in their telemetry pens as pairs of 2 in the early morning. An initial period of 30–45 min allowed the minipigs to acclimate to these measurement pens. After this acclimatization period, the measurements were started. The subsequent hour preceding the administration procedure was taken as a control period for baseline measurements. After this hour, the minipigs were taken out of their pens and vehicle was administered orally (drinking water, volume $1.5 \times$ bodyweight) with a special dosing catheter. In this study for the generation of normal, physiological parameters, only vehicle (water) was administered. After dosing, the minipigs were returned promptly to their telemetry pens and thereafter left undisturbed. The animals were monitored continuously by video for the duration of the study. They had free access to water and 7 h after application they were fed their standard diet.

2.5. Data acquisition and analysis

Digitized telemetry signals were processed by NOTOCORD software (Hem 4.2) on a beat-to-beat basis. The following parameters were continuously measured throughout the experiment: aortic pressure (AP, @250 Hz), left ventricular pressure (LVP, @500 Hz), ECG (@100 Hz) and temperature (@1 Hz). Hemodynamic parameters calculated from these signals during the experiment included: systolic and diastolic aortic pressure, peak systolic and end-diastolic left ventricular pressure, LV dP/dt max and min, heart rate and from the ECG wave PQ-, QRS- and QT-intervals. EXCEL software was used for data analysis. Data were extracted using an Excel® spreadsheet, and presented as the median \pm SD of at least 400 sequential beats at each time point. The mean of the median values \pm SD of every parameter is presented in graphically over 24 h study period. Values after placebo administration were compared to the pre-treatment values. For statistical evaluation, a standardized area under the curve was calculated for each parameter to be evaluated at predefined time intervals. Two measurement phases were defined, one being the lighted period before feeding and the other being the dark period after feeding. The specification of the time interval was as follows:

- 1 h to 7 h after administration of placebo (light period)
- 8.3 h to 20.3 h after administration of placebo (dark period)

Comparisons between these 2 phases were performed by a paired *t* test in 4 animals, from which 8 experiments per individual were available. Statistical significance was accepted when $p < 0.05$.

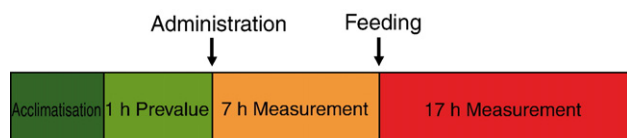


Fig. 1. Study design.

Table 1

Summarized values for measured hemodynamic parameters, heart rate and body temperature in the freely moving Göttingen Minipig

	Mean (day, 1–7 h)	\pm SD	\pm SEM	Mean (night, 8.3–20.3 h)	\pm SD	\pm SEM
SAP mm Hg	122	15	0.43	123	14	0.29
DAP mm Hg	86	10	0.30	88	9	0.18
LVP mm Hg	113	15	0.38	110	14	0.24
LV dP/dt mm Hg/s	2282	390	9.69	2450	288	5.08
HR bpm	56	7	0.23	71	10	0.18
Temp. °C	37.0	0.8	0.02	37.5	1.1	0.02

For evaluation of the QT-RR relationship, a newly inhouse developed computer program (Qtana) was used. Using this program the data were divided into a training set and a test set. With the data from the training set, the regression algorithm calculated the best parameter estimates (a, b, c, d) for each correction function (nonlinear, logarithmic, exponential, Sarma, etc., see Table 3). Different criteria, such as the Pearson's coefficients (*r*), Akaike's information criterion (AIC) and PRESS RMSE were considered in order to select the best model.

To illustrate the results, the QT or QTc values are plotted against RR and the respective correlations were determined and graphically displayed as the corresponding regression lines as described elsewhere (Meyners & Markert, 2004).

For the determination of the power of the statistical test, it was assumed that four groups were under investigation, each consisting of 3, 4, 5 and 6 animals, respectively. It was considered that all groups consist of the same number of animals.

For each parameter, the different base levels of the animals are taken into account by referring the values after administration of the test compound to the pre values (30 min before administration).

With these values the standardized area under the curve (AUC divided by interval length) is calculated. For each compound, two phases are considered, while the specification of the time intervals is arbitrary for this analysis, as the results are valid in all applicable circumstances.

Furthermore, a relevant difference of interest was defined in advance as $\delta = 10$ or 20 percentage points dependent on the target parameter. Hence, the power was given by

$$\text{Power} = 1 - P\left(T \leq t_{1-\frac{\alpha}{2}, \text{edf}}\right) + P\left(T \leq t_{\frac{\alpha}{2}, \text{edf}}\right),$$

where *T* is *t*-distributed with edf degrees of freedom (error dfs from the ANOVA) and non centrality parameter $\sqrt{n \frac{\delta}{\sigma}}$.

The statistical evaluation was performed using the software package SAS Version 8.2.

3. Results

All animals survived the surgical procedure and recovered quickly following the surgery. We obtained excellent signal quality and found stable hemodynamic parameters with a low intrinsic heart rate in the Göttingen Minipig. After oral dosing (in this case with only vehicle/water) the hemodynamic parameters returned quickly to baseline values indicating that the procedure was well tolerated. Interestingly, feeding was associated with a marked and sustained effect on hemodynamic parameters and body temperature. A summary of hemodynamic values, HR and body temperature is given in Table 1.

4. Heart rate (HR)

The course of heart rate values is shown in Fig. 2. The dark period is represented as the shaded area between 8.3 and 20.3 hours. After oral administration values returned to baseline level within 1 h. The fact that heart rates remained below 100 bpm even during the administration indicates that the animals were well-trained and that the procedure was not associated with a high level of stress. During the

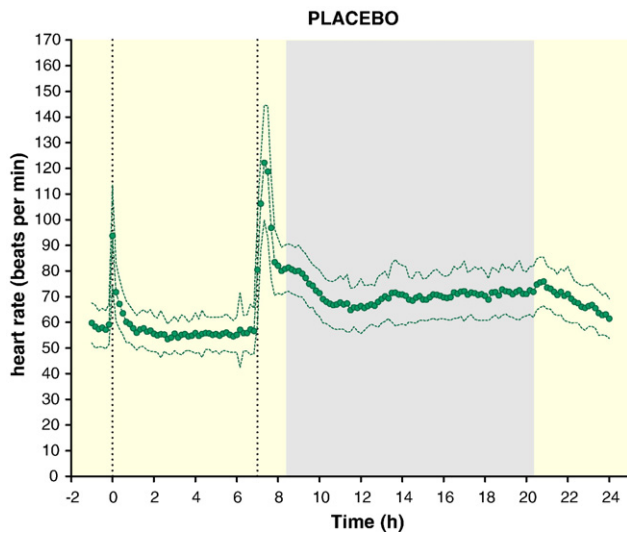


Fig. 2. Heart rate (HR) measured over a 24 h monitoring period with a fully implantable telemetric device (ITS) in freely moving Göttingen Minipigs. Data of all experiments were summarized as mean \pm SD. The dotted line at 0 h indicates oral administration of placebo whereas the dotted line at 7 h indicates feeding. The grey shaded area represents the dark period.

next 7 h of daytime, HR averaged 56 bpm (mean of all experiments) but increased at feeding to 122 bpm. The marked effect of feeding on HR was long-lasting and never returned to the daytime level for the rest of the monitoring period (mean of all experiments at night: 71 bpm). Comparison of the light period (1 to 7 h) and the dark period (8.3 to 20.3 h) showed a highly significant ($p < 0.0001$) increase of heart rate during the night. A further experiment ($n = 4$) was performed without feeding. In this study a mean HR during daytime of 53 bpm was followed by a heart rate of 51 bpm at night (Fig. 3),

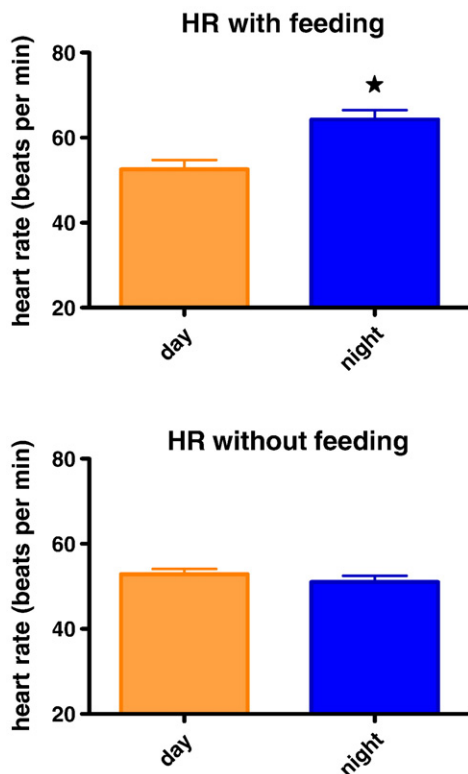


Fig. 3. 1 HR in 4 animals at day (mean 53 bpm) compared to night (mean 64 bpm) with feeding 7 h after treatment; $p = 0.0120$.

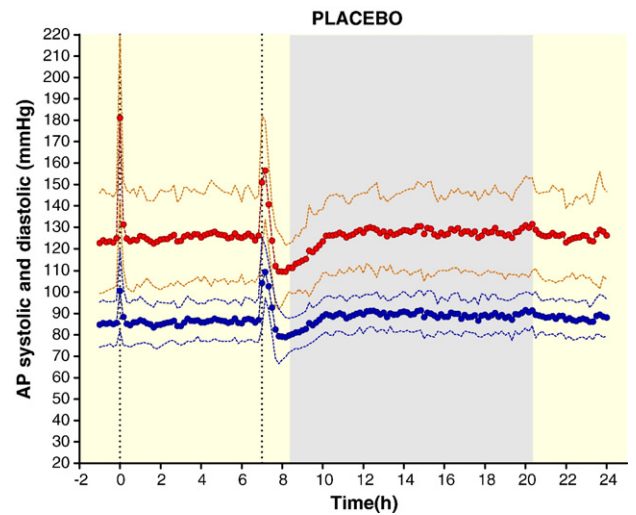


Fig. 4. Systolic and diastolic arterial blood pressures measured over 24 h monitoring period with a fully implantable telemetric device (ITS) in freely moving Göttingen Minipigs. Data of all experiments were summarized as mean \pm SD. The dotted line at 0 h indicates oral administration of placebo whereas the dotted line at 7 h indicates feeding. The grey shaded area represents the dark period.

demonstrating that the effect is dependent upon the postprandial condition and is not merely a nocturnal effect.

4.1. Systolic and diastolic arterial blood pressure

Fig. 4 shows the systolic (SAP) and diastolic (DAP) arterial blood pressure over the 24 h monitoring period. Arterial blood pressure was very stable throughout, with a SAP of 122 ± 16 mmHg and a DAP of 88 ± 10 mmHg. Short-lasting artefacts were associated with the administration procedure and with feeding. No differences in SAP or DAP were seen between day and night phases (SAP, $p = 0.7224$, DAP $p = 0.3793$).

4.2. Systolic left ventricular pressure (LVP systolic)

The left ventricular pressure is summarized graphically in Fig. 5. Aside from the changes at dosing and feeding, the peak systolic left ventricular pressure remained at 111 ± 15 mmHg throughout the

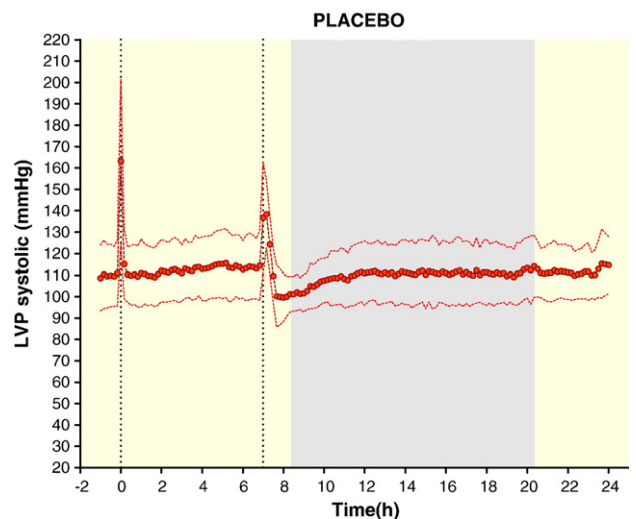


Fig. 5. Peak systolic left ventricular pressure (LVP systolic) measured over a 24 h monitoring period with a fully implantable telemetric device (ITS) in freely moving Göttingen Minipigs. Data of all experiments were summarized as mean \pm SD. The dotted line at 0 h indicates oral administration of placebo whereas the dotted line at 7 h indicates feeding. The grey shaded area represents the dark period.

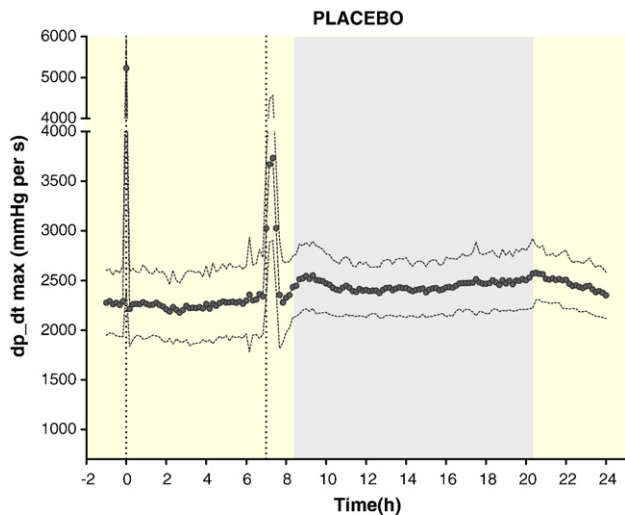


Fig. 6. LVP dp/dt max measured over a 24 h monitoring period with a fully implantable telemetric device (ITS) in freely moving Göttingen Minipigs. Data of all experiments were summarized as mean \pm SD. The dotted line at 0 h indicates oral administration of placebo whereas the dotted line at 7 h indicates feeding. The grey shaded area represents the dark period.

24 h. There was no diurnal rhythm with no significant difference ($p=0.0822$) when the lighted period and the dark period were compared.

4.3. LV dp/dt max

LV dp/dt max was calculated over the monitoring period as a measure of contractility of the left ventricle (Fig. 6). Similar to heart rate, this parameter was significantly ($p<0.0001$) higher at night. In contrast to heart rate, the effect of feeding on LV dp/dt was not that sustained but returned towards baseline levels 1 h after feeding. However, the slight elevation of LV dp/dt throughout the night may be attributed to the prolonged increase in heart rate. Without feeding this finding disappeared and LV dp/dt showed a reversed course with lower levels at night than day ($p=0.0347$).

4.4. Body temperature

Fig. 7 shows the body temperature measured over 24 h. During the day, the pigs had an average temperature of 37.0 °C. In parallel to HR, body temperature increased significantly ($p=0.0011$).

4.5. ECG evaluation

The polarity of the T-wave shifted during the monitoring period without obvious reason. Some examples of ECG waveforms from the same animal in the same experiment are shown in Fig. 8.1–4. However, at higher heart rates (for example after dosing or feeding) the T-wave was positive in all animals. In summary 45% of the T-waves were positive, 42% negative and 13% bipolar. The morphology of the P-wave was typical for each individual and never changed obviously during the course of the experiment.

4.6. ECG values

Values for (manually) measured ECG intervals over the whole course of 24 h in the freely moving Göttingen Minipig are summarized in Table 2.

4.7. QT correction

The heart rate dependency of the QT interval was different for each animal indicating that an individual correction is needed. Fig. 9 shows

the uncorrected QT plot against RR and the QT correction with different formulas for the same individual (based on 1040 observations). In this case, a hyperbolic formula fitted best. In Table 3 the parameter estimates a, b, c and selection criteria as the Pearson's coefficients (r), Akaike's information criterion (AIC) and PRESS RMSE are listed for tested correction formulas.

4.8. Power

Table 4 shows the summarized power calculation values for the parameters measured. With the exception of QT-interval, we found statistically sufficient power in the minipig telemetry to show effects with $n=3$. With $n=4$, however, for all parameters highly statistically sufficient power based on a cross-over experimental design could be shown.

5. Discussion

These are, to our knowledge, the first data documenting the course of systemic arterial and ventricular hemodynamic parameters in the freely moving Göttingen Minipig over 24 h. As such, they may serve as a basis for future studies in which drug effects are studied in these animals.

5.1. Intrinsic heart rate in the Göttingen Minipig

The high level of training as well as the excellent health status of these animals is evident with the low intrinsic heart rate of 56 bpm during daylight hours. Indeed, the low heart rates were surprising if one compares the currently available hemodynamic data in minipigs obtained with invasive measurements, or data from dogs when used for similar studies. Kano et al. (2005) reported values from 72–76 bpm in 17 kg weighing, freely moving miniature pigs (but not Göttingen Minipigs). HR in resting miniature pigs was 80 ± 3.5 bpm in investigations from Kuwahara et al. (1999). Beglinger et al. (1975) report heart rates of 103 ± 14 bpm in sling-restrained Göttingen Minipigs (~20 kg). Thus, we attribute the very low heart rates seen in this study to the low-stress levels achieved using the well-trained animals in a laboratory environment in which measures have been taken to reduce unnecessary stress factors. Furthermore, a low variability of the measured data was seen throughout the study which we also attribute to both training status and study environment. A low variability in the measured data is extremely important

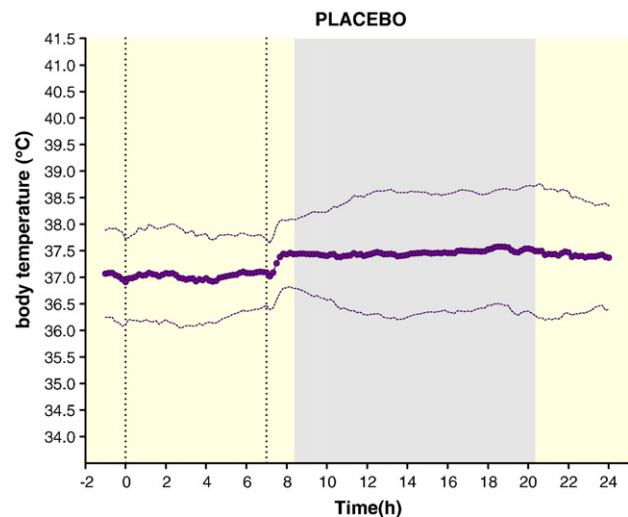


Fig. 7. Body temperature in °C measured over a 24 h monitoring period with a fully implantable telemetric device (ITS) in freely moving Göttingen Minipigs. Data of all experiments were summarized as mean \pm SD. The dotted line at 0 h indicates oral administration of placebo whereas the dotted line at 7 h indicates feeding. The grey shaded area represents the dark period.



Fig. 8. Examples of ECG waveforms in the same animal during the same experiment. 1. T-wave positive. 2. T-wave negative. 3. T-wave \pm . 4. ECG at high heart rates after dosing or feeding.

for the detection and quantification of potential drug-induced effects. Lower variability leads to an optimization of the statistical power of an experimental model and the possibility to reduce the number of animals used. The HRs in the present study are quite comparable to data from freely moving well-trained and group-housed Labrador dogs (Klumpp et al., 2006). This indicates that factors other than the species are decisive for the heart rates observed and this likely reflects the level of acclimatization to the laboratory environment, the low-stress experimental procedure and the fact that no restraint was needed.

5.2. Diurnal and feeding effects on hemodynamics

Diurnal rhythms of heart rate in minipigs have been investigated by Kuwahara, Tsujino, Tsubone, Kumagai, Tsutsumi, & Tanigawa (2004). They found that heart rate in the light phase was higher than in the dark phase when the animals were housed individually, but minipigs housed in pairs had no diurnal variation. Another group (Kano et al., 2005) reported no marked changes in heart rate between light and dark periods. The present data differ from both of these findings: we saw a significant increase in HR at night when the animals were fed shortly before the start of the dark phase. Intuitively, one would expect HR to decrease at night with an increase in parasympathetic activity. Examination of videos taken at night indicate that the minipigs were indeed sleeping most of the time at night (main sleeping period 11 p.m. to 5 a.m.), with only short periods of wandering but with no signs of excitement. When the study design was altered to eliminate feeding at 7 h, the HR at night was 51 bpm being comparable to the daylight value.

These findings indicate a dependency of the dark phase increase in HR on feeding. The pig is known to digest slowly, taking about 53–58 h to empty the digestive tract after a meal, dependent on the kind of diet (Fernandez-Gil, Focant, Elsen, Van Hoccke, & Vanbelle, 1995). A study by Lückmann (1968) investigated HR in domestic swine and reported an increase in heart rate of 8–10% after feeding. The postprandial increase in HR can be larger with voluminous diet (Leucht et al., 1982). This phenomenon may be due to autonomic nervous fibers located in the wall of the digestive tract that react to the filling status of the gastrointestinal tract. Nevertheless, it is clear that the effect of feeding on HR can extend beyond 3 h and that this must be taken into account when designing studies in Göttingen Minipigs.

5.3. Impact of model on hemodynamic parameters

Since blood pressure varies with age and body weight (Beglinger et al., 1975; Gauvin et al., 2006) and various blood pressure

Table 2
Summarized values for (manually) measured ECG intervals during 24 h in the freely moving Göttingen Minipig

	Mean (all time points)	\pm SD	\pm SEM	Mean (HR \leq 80)	\pm SD	\pm SEM
PR (ms)	125	21	0.30	128	22	0.39
QRS (ms)	56	9	0.13	56	9	0.15
QT (ms)	320	38	0.53	335	26	0.46
RR (ms)	861	272	3.81	995	224	3.87

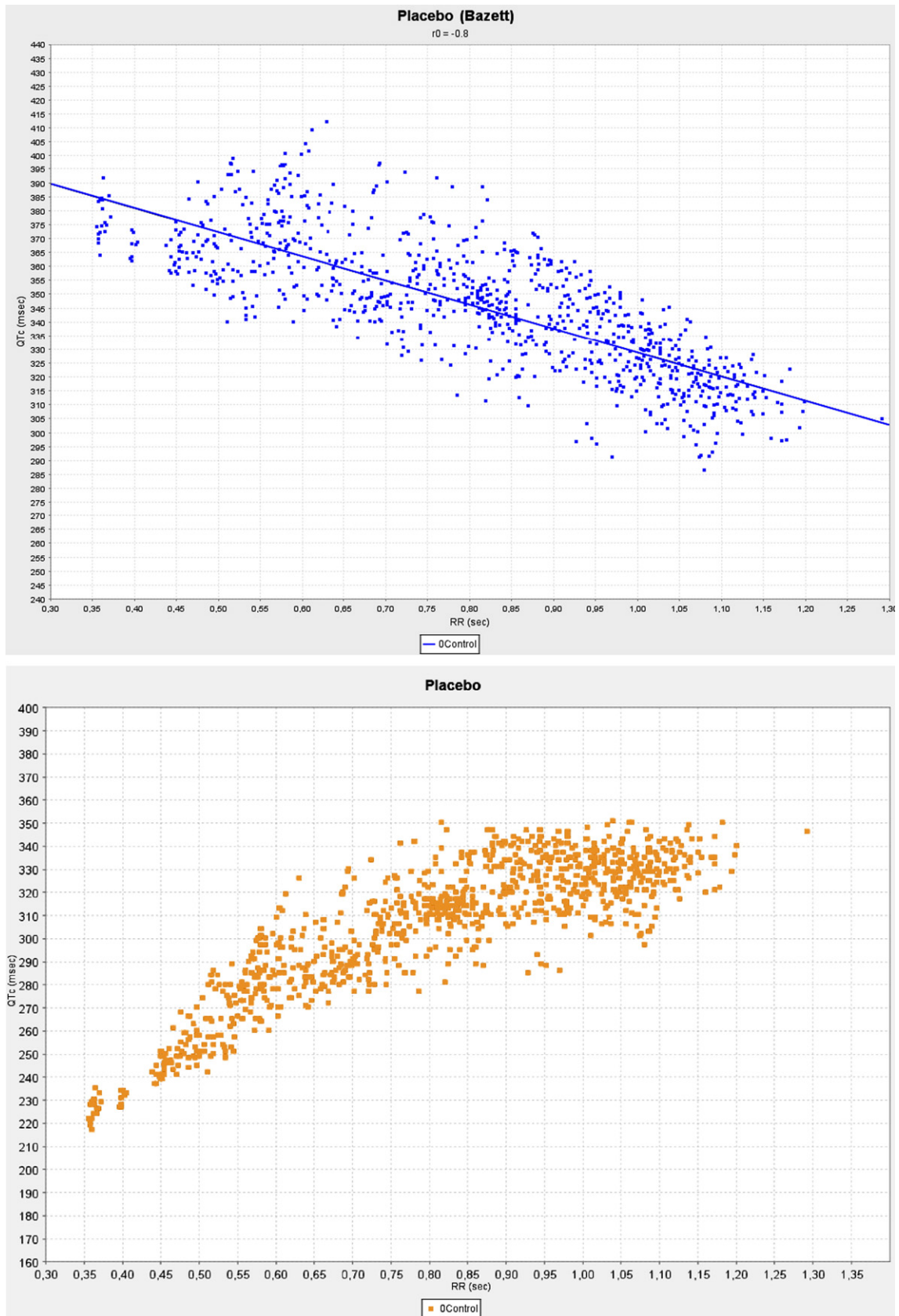


Fig. 9. (1–3) Comparison of QT correction models.

measurement techniques (direct-indirect, awake-anesthetized) are available, comparisons should be done with caution. Furthermore, the present data (122 ± 16 mm Hg and a DAP of 88 ± 10 mm Hg) are

first data coming from freely moving minipigs. Values returned to pre-dose values very quickly (in about 20 min) after dosing indicating that our minipigs were well accustomed to the study

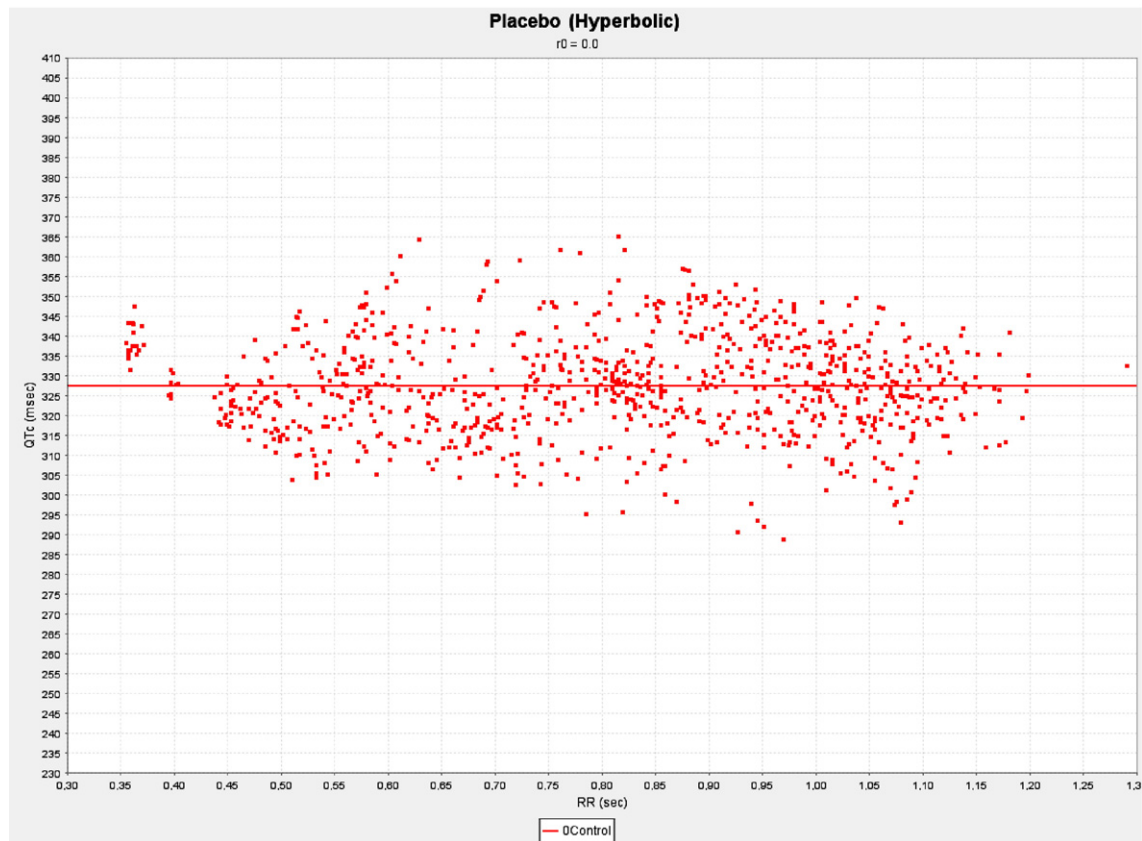


Fig. 9 (continued).

conditions. This is in sharp contrast to the effects observed upon feeding which lasted 3–4 h.

Systolic and diastolic arterial blood pressures were remarkably stable throughout the protocol without a diurnal effect with or without feeding. Values for arterial blood pressure from minipigs range from 135 ± 12 to 160 ± 11 for SAP and 88 ± 4 to 96 ± 14 for DAP (Beglinger et al., 1975; Cimini & Zambraski, 1985; Glodek & Oldigs, 1981; Leucht et al., 1982). None of the previous studies used normal, freely moving minipigs. Thus, the slightly higher pressures reported previously may be attributable to the restraint used when taking the measurements. Left ventricular pressure values in normal, freely moving Göttingen Minipigs are presented in this publication for the first time. Glodek & Oldigs (1981) reported LVP measurements with pressure-tip catheters in awake, but sling-restrained animals and reported a value of $142 \pm$

14 mm Hg. In anesthetized miniature pigs, a LVP of 58 ± 2 mmHg was reported in juvenile Yucatan Miniature swine (Smith et al., 1990) whereas 158.5 ± 36.5 was observed in adult Göttingen Minipigs (Benharkate et al., 1993). This substantial difference is likely explained by the markedly different models used. The mean value measured in our 7 minipigs over 24 h was $111 \pm$ mmHg, a value that remained extremely stable apart from dosing and feeding.

5.4. Diurnal effects on body temperature

The body temperature of the minipigs also demonstrated differences between daytime and nighttime. During daytime (before feeding) body temperature was 37.0 °C in our 7 minipigs, whereas during nighttime it increased to 37.5 °C. The body temperature range

Table 3

Parameter estimates a, b, c and selection criteria Pearson's correlation coefficient r, Akaike's information criterion (AIC) and PRESS RMSE derived from 1040 observations of an individual QT correction

Model	a	b	c	r	AIC	RMSE
Bazett $QT = a * RR^{(1/2)}$	341.31			-0.78	2684.00	73.67
Fridericia $QT = a * RR^{(1/3)}$	330.96			-0.10	2320.60	41.15
Sarma exponential $QT = a - b * exp(c * RR)$	349.36	356.61	-2.82	0.00	2266.03	37.59
Linear $QT = a + b * RR$	206.02	123.59		0.02	2267.05	37.71
Hyperbolic $QT = a + b / RR$	391.56	-63.90		0.00	2264.93	37.58
Parabolic $QT = a * RR^b$	329.43	0.31		0.04	2260.10	37.29
Logarithmic $QT = a + b * log(RR)$	329.41	93.82		-0.03	2269.86	37.88
Shifted logarithmic $QT = log(a + b * RR)$	4.21	1.28		0.73	1814.80	18.27
Exponential $QT = a + b * exp(-RR)$	429.82	-271.97		-0.02	2270.48	37.92

Table 4

Power calculation for the parameters measured with various n numbers

Parameter	Phase	#A	Root MSE	δ	Power			
					(%)	N=3	N=4	N=5
SAP	1	4	3.271731	10	87.4005	96.9134	99.3026	99.8520
DAP	1	4	2.956288	10	92.8689	98.8384	99.8277	99.9762
LVP max	1	4	2.544483	10	97.7260	99.8362	99.9894	99.9994
LV dP/dt max	1	4	4.976622	20	98.1524	99.8852	99.9936	99.9997
Heart Rate	1	4	4.661808	20	99.0587	99.9638	99.9988	100.0000
RR-interval	1	4	5.059347	20	97.8439	99.8505	99.9907	99.9995
PR-Duration	1	4	2.170338	10	99.6032	99.9917	99.9998	100.0000
QRS-interval	1	4	1.480997	10	99.9999	100.0000	100.0000	100.0000
QT-interval	1	4	3.816120	10	76.2381	90.8170	96.6655	98.8477

The dark grey field shows that the power for detecting a 10% change in QT interval is too small with an N of 3 (i.e. <80%).

given in literature for normal miniature pigs ranges from 37–38 °C (Bollen, Hansen, & Rasmussen, 2000), 37.7±0.2 °C (Georgiev, Schoen, & Merckenschlager, 1972) to 38.2–39.9 °C (Zambraski & Fuchs, 1980). It should be mentioned that the temperatures measured using the full implant telemetry system are not actually core temperatures, since the temperature module is in the transmitter of the ITS “T27-F” device located in the abdominal muscle layers of the left flank. The increased heart rate during the night phase may have contributed to the increase in body temperature. But temperature was also increased in the experiment without feeding in which heart rate was not affected, indicating that another factors than the elevated heart rate were responsible. One possibility is that the prolonged periods of lateral recumbency during sleeping may have led to an artifactual effect on temperature, since there was a warming lamp from above and the floor was heated.

5.5. Value of myocardial contractility assessment

The first derivative of LVP over time, LV dP/dt, is a well recognized index of myocardial contractility (Ross, Covell, & Sonnenblick, 1967). Many drugs influence myocardial contractility, hence this parameter gives important information in the evaluation of drug candidates and should therefore be included in testing for cardiovascular side effects. Changes in the inotropic state of the heart can also have effects on systemic arterial blood pressure and cardiac output. There is a clear dependency of LV dP/dt on HR and we have previously described this phenomenon in Labrador dogs, Rhesus monkeys, Cynomolgus monkeys and minipigs (Markert et al., 2007). We saw a difference in LV dP/dt between the light and the dark period which might be related to the observed increase in HR. Values for LV dP/dt similar to that observed in the present study were observed in anaesthetized Göttingen Minipigs (2492±350 mm Hg/s; Benharkate et al., 1993). However, sling-restrained Göttingen Minipigs had higher levels of LV dP/dt presumably due to the higher heart rates and possible stress associated with the restraint (3821±787 mm Hg/s; Glodek & Oldigs, 1981).

5.6. ECG evaluation

Reported ECG investigations in the Göttingen Minipig were done without exception in relatively young (<198 days) and sling-restrained animals (Beglinger et al., 1975; Eckenfels & Schuler, 1988; Glodek & Oldigs, 1981; Hohns, 1970; Nahas, Baneux, & Detweiler, 2002; Jones, Stuart, & Greufe, 1999). Therefore, all the reported values are in much lower ranges than our ECG results (PR 83±8 ms, QT 252±30 ms to 290±22 ms; QRS 36±4 ms to 58±5 ms). The spontaneous changing of the polarity of the T-wave as we saw it, was also apparent in other experiments in Göttingen Minipigs (Eckenfels & Schuler, 1988; Nahas, Baneux, & Detweiler, 2002); the reasons for these spontaneous changes are unclear. We could not, however, see a direct dependency between body position and the T-wave polarity. The T-wave was always positive during periods of excitement in the animals (see Fig. 8.4). The end of T-wave was easy to detect in both positive and negative morphologies (Fig. 8.1 and 2). In contrast, the end of the bipolar T-waves often was difficult to define (Fig. 8.3) and may cause problems for algorithms used for computerized ECG evaluation. The development of new algorithms to cope with this issue is promising.

QT is known to be strongly correlated to the RR interval. Various algorithms have been suggested to describe the relationship between QT and the RR interval or heart rate (Batey & Doe, 2002; Chiang, Holdsworth, & Leishman, 2006; Davey, 2002; Holzgrefe, Cavero, & Gleason et al., 2007; Matsunaga et al., 1997 107, Miyazaki & Tagawa, 2002; Spence et al., 1998; Meyners & Markert, 2004). Based on the present data, we conclude that no correction model is applicable to all individuals, suggesting the use of an individual correction of the QT interval for heart rate changes.

6. Conclusion

For the first time the present study provides values for aortic blood pressure, left ventricular pressure, LV dP/dt, heart rate, body temperature and ECG in the normal, freely moving Göttingen Minipig using a fully implantable telemetric device. We saw extremely stable patterns of all hemodynamic parameters and a good signal quality. In conclusion, the trained Göttingen Minipig appears to be well suited for cardiovascular safety pharmacology studies. Because of the described effect of feeding, we recommend to not feed the animals in studies in which the Tmax of a given compound might occur very late (i.e. after the feeding).

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