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Use of Fiber Optic Technology to Measure the Effects of Anesthesia on Luciferase Reaction Kinetics

Sabrina Semprini,1,† Christopher D Saunter,2,† Mike Ludwig,7 John M Girkin,2 and John J Mullins1,∗

In vivo bioluminescent imaging (BLI) is a sensitive and reliable technique for studying gene expression, although experiments must be controlled tightly to obtain reproducible and quantitative measurements. The luciferase reaction depends on the availability of the reaction substrate, oxygen, and ATP, the distribution of which can vary markedly in different tissues. Here we used in vivo fiber optic technology, combined with stereotaxis-assisted surgery, to assess luciferase reaction kinetics in response to 2 anesthetic regimens, isoflurane and ketamine–xylazine. Transgenic rats that expressed luciferase under the control of the human prolactin promoter were used as a model organism. Anesthesia had a marked effect on luciferase reaction kinetics. The rise time to peak emission differed by 20 min between isoflurane and ketamine–xylazine. Optical imaging using a charge-coupled–device camera confirmed this delay. These results demonstrate that different anesthetics can have substantial effects on luciferase reaction kinetics and suggest that the timing of image acquisition after substrate injection should be optimized in regard to experimental conditions and the tissues of interest.

Abbreviations: BLI, bioluminescent imaging; CCD, charge-coupled device; ROI, region of interest.

Luciferase from Photinus pyralis is used widely as a reporter gene for in vivo bioluminescence imaging (BLI). Luciferase is an enzyme that can generate visible light through the oxidation of its substrate, D-luciferin, in the presence of oxygen and ATP.13 This enzyme has been used to tag bacteria and mammalian cells, including tumor and immune cells, and in the generation of transgenic animals for in vivo studies of gene expression.24 Typically the light transmitted through the animal’s tissues is detected by using charge-coupled–device (CCD) cameras to provide very sensitive in vivo measurements.4 To achieve quantitative measurements, the experimental conditions need to be considered carefully, with great attention to all parameters that might influence the reaction kinetics, light output, and consistency of the measurements.

Anesthesia is known to interfere with many physiologic processes, particularly cardiopulmonary functionality,29 which might influence substrate biodistribution. Consequently, anesthesia might affect photon emission in BLI experiments and needs to be evaluated carefully when planning the use of bioluminescence during in vivo experiments. Luciferase itself has served as a useful model to demonstrate a direct interaction between general anesthetics and proteins at the molecular level,9,10,18,21,25 showing that a wide range of anesthetics at different potencies compete with D-luciferin for binding to the luciferase enzyme. The result of this interaction is an inhibition of the luciferase enzyme and a subsequent reduction of the total light output. The effects of anesthesia on the kinetics of the luciferase reaction are not well characterized. After intraperitoneal injection of D-luciferin, the kinetic profile of the luciferase reaction usually shows a gradual increase of photon emission, a peak with a stable plateau followed by a slow decrease in photon emission.30 The identification and repeatability of the stable plateau of light emission in any experimental condition is crucial for maintaining the quantitative nature of the measurements.

Here we describe the effects of 2 different anesthetics on the kinetic profile of the luciferase reaction. We used a transgenic rat model (PRL-Luc) that expresses luciferase under the control of the human prolactin promoter in the pituitary gland and various extrapituitary sites, such as bone and cartilage,29 and we performed in vivo intensity measurements by using both a custom fiber-optic probe that was implanted directly into the pituitary gland and a CCD camera. We show that the method of anesthesia has a marked effect in determining the rise time of light output in the luciferase reaction kinetics, and this effect directly influences the timing of image acquisition after substrate injection.

Materials and Methods

Rat model. The generation of PRL-Luc transgenic rats has been described previously.25 Briefly, the transgenic rats were generated by using a BAC clone that included approximately 160 kb of the human prolactin genomic locus. The transgene was microinjected into the pronucleus of Fisher 344 rat zygotes. This study was completed under a UK Home Office License, after review by the local ethics committee. All rats were housed individually in a pathogen-free environment, given free access to water and standard commercial rat chow (Special Diet Service, Witham, UK), and maintained under controlled conditions of temperature (21 ± 1 °C) and humidity (50 ± 10%) under a 12:12-h light:dark cycle. All of the procedures were terminal, and rats were euthanized by CO2 inhalation.

Probe construction. We constructed a fiber-optic device for light collection. The device consists of a 243-µm (outer diameter) silica fiber with a 200-µm core (Polymicro Technology, Phoenix,...
Fiber optic implantation and imaging modality. Female rats (age, 3 mo; weight, 200 g) were anesthetized by using 2 different anesthetics on the kinetics of the luciferase reaction in vivo. We then injected a saturating solution of d-luciferin (50 mM d- luciferin in physiologic saline; 180 mg/kg body weight IP) and imaging started (time 0). The rats were imaged continuously for as long as 1 h. Body temperature was measured by using a rectal probe and was maintained throughout the experiment by using a heating pad.

**Data collection.** Light gathered by the probe was cast onto a photomultiplier tube with self-contained electronics (Hamamatsu Photonics, Hamamatsu City, Japan). The digital signal from this module representing the photon flux was digitized by using a Nexys2 FPGA module (Digilent, Pullman, WA) and recorded at 10-Hz intervals by using a laptop. All data recording occurred in a dark environment to prevent stray light from affecting counts. In each case, we verified that the blood flow and isoflurane curve after 22 ± 0.05 min for isoflurane and 11 ± 0.05 min for ketamine–xylazine (P < 0.05). The length of the plateau is highly significantly (P = 0.0002) different between ketamine–xylazine and isoflurane (9.33 ± 0.33 and 15.33 ± 0.33 min, respectively).

Both curves show a consistent decrease in signal in the last 30 min of observation (33% for ketamine–xylazine and 35% for isoflurane), whereas the signal seems to be more stable when imaged with the fiber optics (Figure 1B).

**Results**

A fiber-optic probe was placed on the dorsal surface of the pituitary of PRL-Luc transgenic rats by using stereotactic surgery, and light-recording data were collected for as long as 1 h by using a photomultiplier tube (Figure 1A). Luciferase reaction kinetics showed a marked difference between the 2 conditions described. Averaged data (Figure 1B) show that the ketamine–xylazine curve peaks at 11 ± 1 min, whereas the isoflurane curve peaks at 31 ± 2 min (P < 0.01). These data were confirmed by using an alternative in vivo imaging method. Images of pituitary and bone–cartilage signals were collected by using a CCD camera (Figure 2A). A clear shift and delay in the isoflurane kinetics is evident in the isoflurane curve compared with the ketamine–xylazine curve (Figure 2B), confirming the data obtained by using the fiber-optic probe in the pituitary. Quantification of the signal showed a 8-min difference between the anesthetics in reaching peak signal, with the ketamine–xylazine curve reaching it after 14 ± 1.7 min and the isoflurane curve after 22 ± 1 min (P < 0.05). Defining the plateau, as the time during which the signal is greater than or equal to 95% of peak value, the time needed to reach center plateau is 16.33 ± 1.86 min for ketamine–xylazine and 22.67 ± 0.66 min for isoflurane (P < 0.05). The length of the plateau is highly significantly (P = 0.0002) different between ketamine–xylazine and isoflurane (9.33 ± 0.33 and 15.33 ± 0.33 min, respectively). Both curves show a consistent decrease in signal in the last 30 min of observation (33% for ketamine–xylazine and 35% for isoflurane), whereas the signal seems to be more stable when imaged with the fiber optics (Figure 1B).

**Discussion**

The aim of the present study was to investigate the effects of 2 different anesthetics on the kinetics of the luciferase reaction in vivo in rats. We used stereotaxis-assisted surgery to place a fiber optic directly on the pituitary of transgenic rats that express luciferase under the control of the human prolactin promoter.26 This method is sensitive, and the light detection is direct, spatially localized and not perturbed by tissue absorption and scattering, as is the situation for whole-body in vivo imaging. The use of fiber optics in preclinical studies is...
becoming quite widespread, reflecting the need of high resolution measurements and images that can detect changes at the molecular level in different tissues and organs.\textsuperscript{1,6,19} We have demonstrated that anesthesia has a profound effect on the profile of photon emission in rats, introducing variability in the rise time needed to reach peak value and have confirmed these findings by using a CCD camera. In addition to its effects on the rise time, anesthesia can modify the length of the stable plateau. These anesthesia-associated effects have important consequences regarding the reproducibility of quantitative measurements by BLI.

Quantitative BLI is a technique that has increasingly been used to study reporter gene activity in vivo. Most of the applications of BLI require precise quantitative measurements, which are achieved by closely controlling the experimental conditions and the multiple factors that alter photon emission.\textsuperscript{3,16} Anesthetics have been recognized to interact at the molecular level with the luciferase enzyme and to compete with substrate binding to reduce total light production.\textsuperscript{9} We show here that the type of anesthetic can profoundly affect the time needed for the luciferase reaction to achieve peak emission and be maintained within a stable plateau. A 20-min difference has been observed in the time to peak signal between ketamine–xylazine and isoflurane anesthesia when fiber optics have been used to directly quantify light emission from the pituitary gland. This observation has been confirmed by using in vivo BLI, a less invasive imaging method, which has shown an 8-min difference in the rise time to peak signal. In addition, a significant difference was observed in the length of the stable plateau between the 2 anesthetics (9.33 ± 0.33 and 15.33 ± 0.33 min with ketamine–xylazine and isoflurane, respectively).

Isoflurane is administered by inhalation and is the most commonly used anesthetic for in vivo imaging because it is eliminated efficiently by the lungs allowing a rapid recovery. Because it is important to take bioluminescence images either at peak emission or during the plateau of photon emission to ensure that images are quantitative and reproducible at different time points, our study shows that peak time under isoflurane is reached not earlier than 20 min after D-luciferin injection. In the current study, both fiber optic and in vivo optical imaging proved to be very reliable techniques in detecting the rise time of luciferase reaction kinetics but seem to show differences in signal decay. This effect can be explained with a temperature difference between the 2 imaging settings. During optical imaging with the CCD camera, we placed the rats in a closed imaging chamber on a heated pad, which maintained the animals’ body temperature at least 1 °C higher than that during fiber optic imaging. Moreover, consistent heat loss through the fiber-optic probe and the exposed skull in the stereotaxis setting should be anticipated and would reduce the rate of D-luciferin uptake and the speed of the luciferase reaction.

D-luciferin uptake kinetics and biodistribution can vary substantially between different organs,\textsuperscript{3} and the imaging conditions should be validated carefully for the system of interest. To widen the applicability of our observation to the imaging of other organs and tissues, we studies the luciferase reaction kinetics in bone and cartilage tissues in the same rat model by using in vivo optical imaging. In these tissues, the time difference to reach peak light emission was much less pronounced (2 min) with the use of ketamine–xylazine or isoflurane. The anesthetic-associated difference was instead in the absolute photon emission, with signals being 3 times higher with isoflurane as with ketamine–xylazine. To explain these differences, the effects on the luciferase reaction of metabolic characteristics of the anesthetics and the hypoxic environment of cartilage need to be considered.\textsuperscript{25} Anesthetic agents and their carrier gases have well-known potential to alter tissue oxygenation.\textsuperscript{12,14} We therefore speculate that, given the highly hypoxic environment of cartilage, administration of isoflurane in oxygen would increase the oxygenation status of this tissue. Because the luciferase reaction is highly dependent on oxygen availability, isoflurane anesthesia could increase the total photon emis-
Anesthesia affects luciferase reaction kinetics in vivo could profoundly influence the interaction of the anesthetic with the luciferase enzyme.

Given that our model expresses luciferase under the control of the prolactin promoter and that anesthesia can influence prolactin secretion,7,17,22 we are aware that quantitative effects on the levels of luciferase expression could be dependent on the model. However, we believe that observations on the variations of time to reach peak level of photon emission or the length of the plateau are of general validity in making scientists aware of the importance of characterizing the chosen anesthetic in their experimental setting.

The results of the current study demonstrate a clear effect of anesthesia on luciferase reaction kinetics, which are also dependent on the site of imaging and strengthen the need for carefully choosing and testing the experimental conditions before any bioluminescence in vivo imaging analysis. This study identifies anesthesia as a parameter, which is critical for robust quantitative measurements during in vivo optical imaging.

Our data also strengthen and confirm previous findings about the alteration of luciferase reaction kinetics under different anesthetic regimes.5,15 In light of our results, we strongly suggest the performance of a pilot study to determine the time kinetics of the luciferase reaction under the anesthetic regime chosen.

Table 1. Peak light signal (%; mean ± SE) during ketamine–xylazine (KX) or isoflurane anesthesia calculated by using either luciferase fiber-optic imaging or optical in vivo bioluminescence imaging (BLI) data

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Fiber optic</th>
<th>BLI</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>KX (n = 7)</td>
<td>Iso (n = 3)</td>
</tr>
<tr>
<td>5</td>
<td>68 ± 14</td>
<td>37 ± 6</td>
</tr>
<tr>
<td>10</td>
<td>87 ± 10</td>
<td>65 ± 9</td>
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<td>15</td>
<td>93 ± 4</td>
<td>77 ± 8</td>
</tr>
<tr>
<td>20</td>
<td>91 ± 5</td>
<td>85 ± 7</td>
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Table 2. Optical imaging of luciferase kinetics in PRL-Luc transgenic rats expressing luciferase in the pituitary and bone–cartilage. (A) Image of a transgenic rat by using the IVIS spectrum. Pituitary and bone–cartilage (paw) regions of interest (ROI) have been marked and analyzed in regard to dynamic light emission. (B) Luciferase kinetics curve of pituitary light emission (ROI 1) after anesthesia with ketamine–xylazine (K/X; red; n = 3) and isoflurane (Iso; blue; n = 3). Data were not normalized. Inset: raw, single-rat data. (C) Luciferase kinetics curve of bone–cartilage light emission (ROI 2) after anesthesia with ketamine–xylazine (K/X; red; n = 3) and isoflurane (Iso; blue; n = 3). Data were not normalized. Inset: raw, single-rat data.

Figure 2. Optical imaging of luciferase kinetics in PRL-Luc transgenic rats expressing luciferase in the pituitary and bone–cartilage. (A) Image of a transgenic rat by using the IVIS spectrum. Pituitary and bone–cartilage (paw) regions of interest (ROI) have been marked and analyzed in regard to dynamic light emission. (B) Luciferase kinetics curve of pituitary light emission (ROI 1) after anesthesia with ketamine–xylazine (K/X; red; n = 3) and isoflurane (Iso; blue; n = 3). Data were not normalized. Inset: raw, single-rat data. (C) Luciferase kinetics curve of bone–cartilage light emission (ROI 2) after anesthesia with ketamine–xylazine (K/X; red; n = 3) and isoflurane (Iso; blue; n = 3). Data were not normalized. Inset: raw, single-rat data.
This preliminary experiment might be achieved by acquiring sequential imaging after d-luciferin injection and determining the key window for stable measurements or the peak time for measurements, if the plateau time is too short.

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References