Journal of Pharmacological and Toxicological Methods 59 (2009) 7–12

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Original article

A novel type of implantable and programmable infusion pump for small laboratory animals

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ARTICLE INFO

Article history:
Received 17 March 2008
Accepted 9 September 2008

Keywords:
iPRECIO™
Implantable
Programmable
Refillable
Infusion pump
Arterial pressure
Angiotensin II
Losartan

ABSTRACT

Introduction: The iPRECIO™ (Primetech Corporation, Tokyo, Japan) is a new form of pump for infusing small laboratory animals. The key features of the iPRECIO™ are that it can be implanted within the animal, it is refillable, and it is programmable. The infusion start-points and end-points are adjustable, infusion rate can be altered, and the infusion solution can be changed after the pump is implanted. In order to confirm the precision of the iPRECIO™, *in vivo* and *in vitro* experiments were employed. **Methods:** In the *in vitro* experiment, at the excretion rate of 1 μl/h for 336 h, 15 μl/h for 96 h, and 30 μl/h for 120 h, the decrease in each pump weight was used to estimate the actual excretion volume. In the *in vivo* experiments, the iPRECIO™ was chronically implanted in rats, angiotensin II was infused, and arterial pressure (AP) was monitored. **Results:** In the *in vitro* experiment, the volume of solution excreted from the pump increased with time, and the volume excreted matched the programmed volume. The infusion rate also changed at the scheduled time. In the *in vivo* experiment, AP increased and decreased on schedule, and a dose-dependent pressor response to angiotensin II occurred. Furthermore, after exchanging saline with angiotensin II, AP increased and decreased on schedule. **Discussion:** Present data of the *in vitro* and *in vivo* experiments indicates that the iPRECIO™ worked precisely, making it suitable for a variety of experiments involving small laboratory animals.

1. Introduction

Infusion pumps are used to examine the physiological and pharmacological properties of biological or pharmacological substances *in vivo*. Two different types of infusion pumps exist: syringe pumps and implantable pumps. Each of these pumps has its advantages and disadvantages. The syringe pump comprises an external syringe that is connected to a catheter from the animal. One advantage of this design is that it is easy to alter the infusion rate and the infusion solution, although the connection between the syringe and the animal restricts movement and is stressful for the animal. Furthermore, a device is required to prevent tangling of the catheter (Li, Dale, Hasser, & Blaine , 1996; Matsumura, Kinoshita, Satoh, Osaka, & Hayaishi, 1995; Morita, Tsunooka, Hagiike, Yamaguchi, & Lee, 1998). Implantable pumps such as osmotic pumps (Alzet Osmotic Pumps Company, Cupertino, CA) are inserted as a complete unit into small animals. One advantage of this system is that it allows continuous infusion of solutions at a constant rate (Cooper, Heppert, Davies, & Lunte, 2007; Wang, Shamloul, Wang, Meng, & Wu, 2006). In addition, implantable pumps do not restrict the animal's movement, and are therefore less stressful. One disadvantage of this system, however, is that the infusion rate or infusion solution cannot be altered during an experiment.

The iPRECIO™ (Primetech Corporation, Tokyo, Japan) is an infusion pump for infusing small laboratory animals. The key features of the iPRECIO™ are that it can be implanted within the animal, it is refillable, and it is programmable. These features provide the benefits of syringe pumps and other implantable pumps combined in one unit. We performed both *in vitro* and *in vivo* experiments to test the precision and utility of the iPRECIO™. During *in vitro* experiments, we examined the precision of the infusion rate; during *in vivo* experiments, we investigated the suitability of the iPRECIO™ for use with small laboratory animals. For the *in vivo* experiments, we infused angiotensin II at various infusion rates while measuring changes in arterial pressure (AP). As the half-life of angiotensin II is short (Al-Merani, Brooks, Chapman, & Munday, 1978), AP responds rapidly to changes in the infusion rate of angiotensin II. We compared changes in AP with the scheduled infusion time.

2. Materials and methods

2.1. Configuration of the iPRECIO™

The pump comprises a septum, reservoir, pump drive-compartment (houses the micro-motor and cam), input/output port (I/O port), and an activation button (Fig. 1). Solutions introduced via the septum are held in the 900 μl capacity reservoir. For programming, the pump is placed into a

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doi:10.1016/j.vascn.2008.09.002

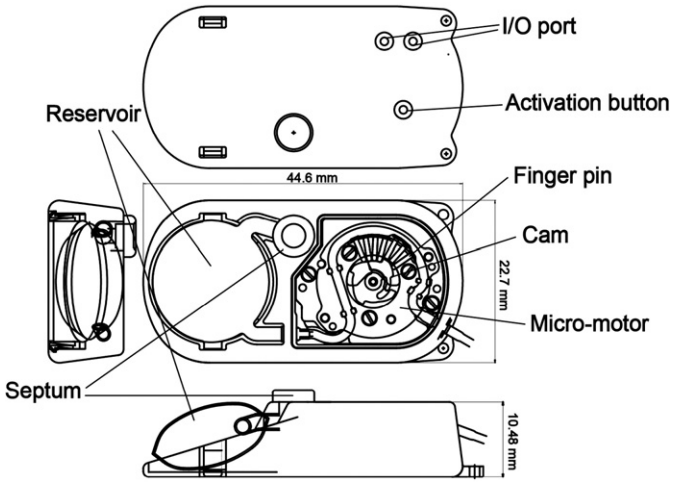

Fig. 1. Configuration of the iPRECIO™ unit. The bottom view is shown in the upper panel, the top view in the middle panel, and the side view in the lower panel. The mass of the iPRECIO™ unit is 10 g.

holding device by pushing the I/O port terminals onto the needle-like contacts of the holding device. This procedure enables access to the internal circuit of the pump. The activation button is pushed to set the pump to a ready state. The pump is implanted subcutaneously, thereby providing access to various administration routes (e.g., subcutaneous, intraperitoneal, intravenous, and intracerebroventricule). The pump also houses a septum designed for percutaneous access, through which solutions are injected or exchanged (upper panel of Fig. 2).

The pump drive system employs the "Rotary Finger Method™" developed and patented by Primetech (lower panel of Fig. 2). In the drive-compartment for the pump, a micro-motor slowly revolves in a clockwise direction turning a cam with its four projections. In each quarter-rotation, a single cam projection sequentially pushes up each of the seven finger pins. This continuous cycle compresses the liquid-filled tube, creating a peristaltic-like movement of the fluid. As the solution moves through the tube, it is forced from the reservoir of the pump into the animal.

2.2. Protocol of the in vitro experiment

This experiment was performed using 13 pumps programmed with the same infusion protocols: 1 µl/h for 336 h, 15 µl/h for 96 h, and 30 µl/h for 120 h. The infusion protocols were programmed with the iPRECIO™ Management Software Ver. 1.1 Rev. 348 (Primetech Corporation, Tokyo, Japan). A water-filled container, pressurized to 60 cm H_2O with the water column (Fig. 3), was used to simulate the *in vivo* environment. The 13 pumps were placed into the water-filled container at 38 °C. The initial pump weights (inclusive of 900 µl saline) were measured using a digital balance; changes in pump weight were used to evaluate the flow rate of each pump. Taking the density of saline into account, the decrease in pump weight was used to estimate the actual excretion volume of the pumps. Each pump was carefully blow-dried to remove any moisture. Each pump weight was measured twice at two different times each day. At the flow rates of 15 and 30 µl/h, the pump was refilled with saline warmed to 38 °C before the reservoir emptied. After refilling, the new weight was set as the 'initial' pump weight until the next refilling.

2.3. Protocol of the in vivo experiments

These experiments were performed on male Sprague–Dawley rats weighing 340–360 g (n=10). Animals used in the present study were maintained in accordance with the "Guiding Principles for Care and Use of Animals in the Field of Physiological Science" set by the Physiological Society of Japan. The experiments were approved by the Animal Research Committee of Gifu University.

The timetable of the experiments is shown in Fig. 4. In Experiments 1 and 3, a pressure transmitter was implanted into the abdominal aorta 1 week before the experimental day for measuring AP. Rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). The catheter part of the pressure transmitter probe (TAP11PA-C40, Data Sciences International, St. Paul, MN) was inserted into the abdominal aorta via central laparotomy. The tip of the catheter was set distal to the renal artery bifurcations. The probe was then sutured to the abdominal wall and the incision was closed. The rats were given penicillin G potassium (6000 U/day) intramuscularly for 3 days and monitored to ensure that food and water intake returned to pre-surgical levels.

In Experiments 1 and 3, the iPRECIO™ was implanted 1 week after implantation of the pressure transmitter. One pump was implanted for Experiment 1 and two for Experiment 3. The infusion start time, infusion end time, and infusion rate were programmed using the software (Table 1). The following solutions (900 µl) were then injected using 27 G needle via the septum: angiotensin II (50 µg/ml) for Experiment 1 (n=4), and angiotensin II (50 µg/ml) and losartan (50 mg/ml) for Experiment 3 (n=2). After programming the iPRECIO™, rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). The catheter part of the iPRECIO™ was inserted into the external jugular vein via a mid-cervical incision. The main body of the iPRECIO™ was implanted subcutaneously under the back of the neck. The second iPRECIO™ in Experiment 3 was

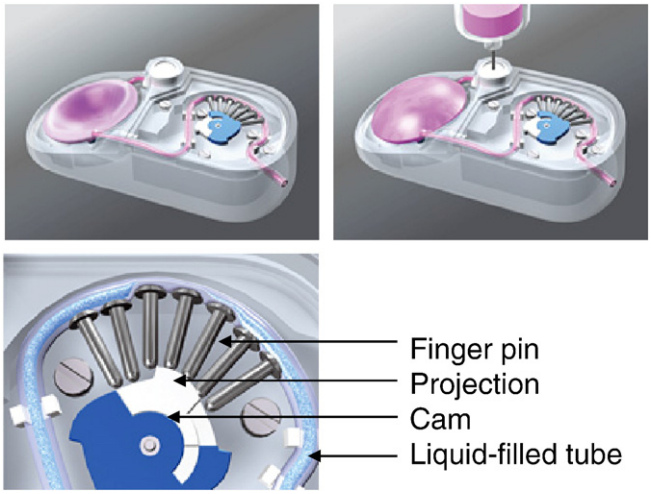

Fig. 2. Upper panel: the illustration shows how to refill or exchange drug solutions. Lower panel: the illustration shows the "Rotary Finger Method™".

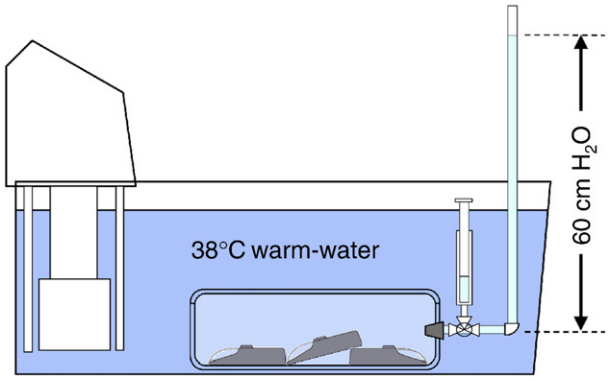

Fig. 3. Illustration of the pressurized container in the water bath. The water column was connected to the water-filled container and adjusted with a three-way valve and a syringe.

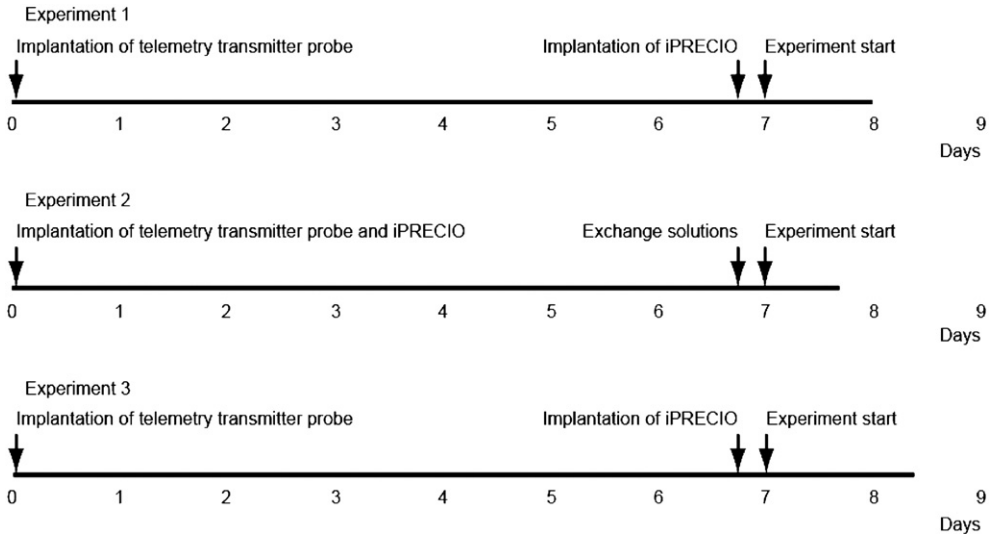

Fig. 4. Timetables of *in vivo* experiments.

implanted into the left femoral vein. After closing the incision, penicillin G potassium (6000 U/day) was injected intramuscularly. All rats were maintained in their individual cages to recover from the surgery until the experiment.

In Experiment 2, the pressure transmitter and iPRECIO™ were implanted on the same day (n=4). The saline was infused during the following 1-week (168 h) recovery period. Seven days after the surgery, the solution in the iPRECIO™ was changed from saline to angiotensin II (50 μg/ml). This procedure was conducted under light anesthesia using enflurane (Abbott Japan, Osaka, Japan) inhalation applied through a face mask. The septum was palpable transcutaneously, and the solution was exchanged using 27 G needle. AP was continuously recorded for 15 h thereafter.

On the day of the experiment, the signal from the AP transmitter was detected by a PhysioTel Receiver (RLA1020, Data Sciences International, St. Paul, MN). The signal was then transformed to an AP signal by a Calibrated Pressure Output Adapter (R11CPA, Data Sciences International, St. Paul, MN) by comparison with the ambient pressure using a Dual Ambient Pressure Monitor (C11PR, Data Sciences international, St. Paul, MN). The signal from the AP transmitter was recorded by an analog-to-digital converter (PowerLab; ADInstruments, New South Wales, Australia) at a rate of 100 Hz. AP was continuously recorded until the iPRECIO™ program stopped.

Table 1
Experimental procedures employed in Experiments 1–3

Experiment 1 (n=4)			
Angiotensin II (50 μg/ml)			
Start time	End time	Duration (h)	Infusion rate (μl/h)
17:00	20:00	3	0
20:00	23:00	3	10
23:00	2:00	3	0
2:00	5:00	3	20
5:00	11:00	6	0
11:00	14:00	3	30
14:00	17:00	3	0

Experiment 2 (n=4)			
Saline			
Start time	End time	Duration (h)	Infusion rate (μl/h)
10:30	10:30	168	3
Exchange solution from saline to angiotensin II			
Angiotensin II (50 μg/ml)			
Start time	End time	Duration (h)	Infusion rate (μl/h)
12:00	15:00	3	30
15:00	18:00	3	0
18:00	21:00	3	20
21:00	0:00	3	0
0:00	3:00	3	10

Experiment 3 (n=2)							
Angiotensin II (50 μg/ml)				Losartan (50 mg/ml)			
Start time	End time	Duration (h)	Infusion rate (μl/h)	Start time	End time	Duration (h)	Infusion rate (μl/h)
12:00	15:00	3	0	12:00	15:00	3	0
15:00	18:00	3	20	15:00	18:00	3	0
18:00	21:00	3	0	18:00	21:00	3	0
21:00	0:00	3	20	21:00	0:00	3	0
0:00	12:00	12	0	0:00	12:00	12	30
12:00	18:00	6	20	12:00	18:00	6	30
18:00	21:00	3	0	18:00	21:00	3	0

2.4. Data analysis

In Fig. 5, a simple linear regression line was calculated using the least squares. In Fig. 8, the data are presented as means±SE, and one-way ANOVA was applied. If the F ratio indicated statistical significance, the Student–Newman–Keuls post hoc test was used. The significance level was set at $P<0.05$.

3. Results

Data from the *in vitro* experiment are shown in Fig. 5. The excreted volume increased with time elapsed. The slope–which represents infusion speed–changed according to the programmed schedule (upper panel of Fig. 5). Simple linear regression plots of the actual volume versus scheduled volume in each infusion rate are shown in the lower panel of Fig. 5. Six hundred and eleven plots were obtained from the 1 μl/h infusion, 182 from the 15 μl/h infusion, and 156 from the 30 μl/h infusion. The fit of the regression was significant for all data, and the measured volume closely matched the scheduled volume.

Fig. 6 illustrates typical changes in AP and heart rate (HR) in Experiment 1. AP increased and HR decreased during the infusion of angiotensin II. AP started to increase according to the programmed schedule at the infusion rates of 20 and 30 μl/h; however, at the infusion rate of 10 μl/h, AP started to increase 5 min after the scheduled time.

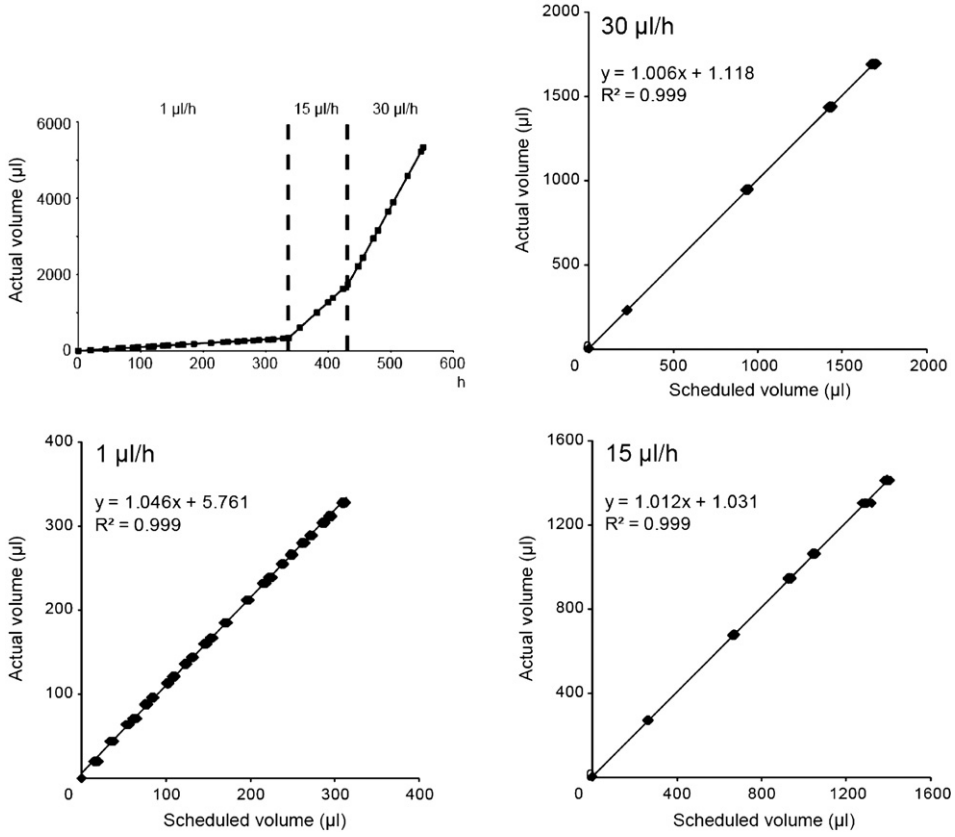

Fig. 5. Upper panel: changes in measured volume with elapsed time. Lower panel: simple linear regression plots of the actual versus scheduled volume for each infusion rate.

Fig. 7 shows the AP response in two of four rats in Experiment 2, in which saline was infused at a rate of 3 µl/h for 168 h, after which saline was replaced with angiotensin II. At the infusion rates of 20 and 30 µl/h, the time point at which AP increased or decreased coincided with the programmed schedule, even after the exchange of solutions. In contrast, at the infusion rate of 10 µl/h, AP increased 5 min later than the scheduled start time, although it decreased at the scheduled time. It was therefore possible to replace the infusion solution after implanting the iPRECIO™, and the iPRECIO™ followed the programmed schedule following replacement of the solutions.

Fig. 8 shows summary of the pressor responses obtained in Experiments 1 and 2. The pressor response was calculated as the difference between the average AP 1 h before the start of infusion and that during the last hour of infusion. The pressor response at the infusion

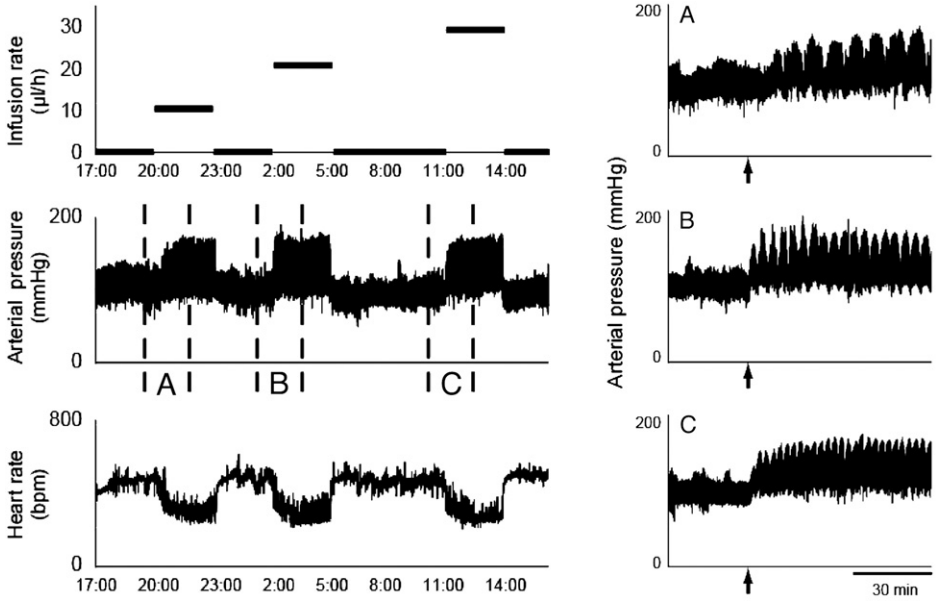

Fig. 6. Left panel: typical changes in AP and HR in Experiment 1. Horizontal bars indicate the period of angiotensin II (50 µg/ml) infusion. Right panel: the time scale of AP changes is expanded. A: 10 µl/h; B: 20 µl/h; C: 30 µl/h.

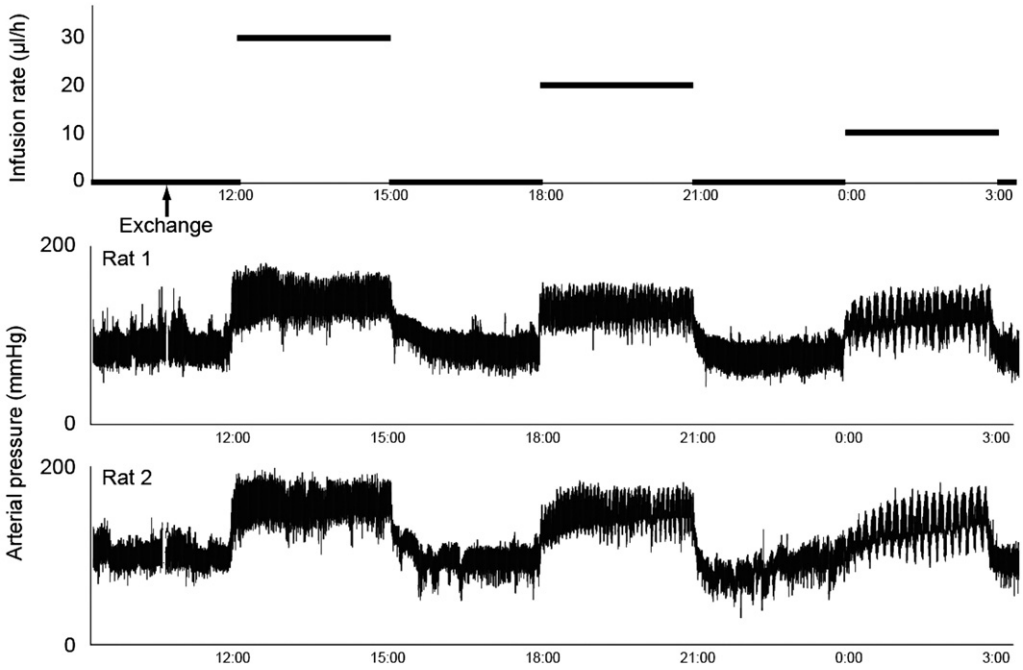

Fig. 7. Changes in AP in Experiment 2. Horizontal bars indicate the period of angiotensin II (50 μg/ml) infusion.

rates of 20 and 30 μl/h was significantly greater than that at 10 μl/h, while the pressor response at 30 μl/h was significantly greater than that at 20 μl/h. We could not confirm the precision of the infusion rate in these *in vivo* experiments; however, we did observe that the AP response was dose-dependent.

Fig. 9 shows changes in AP in Experiment 3, in which two iPRECIO™ units were simultaneously implanted into the rat. AP increased and decreased at the scheduled times. The pressor response occurred during angiotensin II infusion; however, the AP baseline decreased after infusion of losartan, and the pressor response induced by angiotensin II did not occur during losartan infusion. These data indicate that it is possible to implant two iPRECIO™ units in one small laboratory animal.

4. Discussion

We performed both *in vitro* and *in vivo* experiments to test the precision and utility of the iPRECIO™. During *in vitro* experiments, the iPRECIO™ precisely followed the programmed infusion rate and work time, even under conditions of 60 cm H_2O pressure. Based on the results of this *in vitro* experiment, we examined the utility of the iPRECIO™ for *in vivo* experiments involving rats. Because angiotensin II is a rapid and short-acting pressor agent, AP starts to increase soon after the start of intravenous infusion of angiotensin II and decreases soon after the end of infusion (Stocker, Schiltz, Sved, 2004; Zhang et al., 2004). Therefore, the start and end of infusion can be estimated by measuring AP.

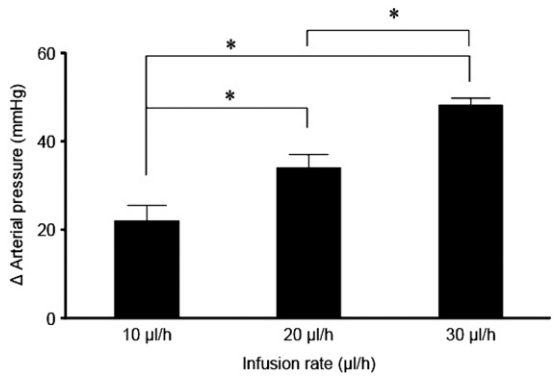

Fig. 8. Summary of the pressor responses in Experiments 1 and 2. *$P<0.05$, $n=8$.

At the infusion rate of 20 and 30 μl/h, AP increased just at the programmed schedule; however 5 min delay was observed at the infusion rate of 10 μl/h (Figs. 6 and 7). This delay is unlikely to have arisen from malfunction of the pump; rather, it is more likely to reflect the time elapsed before becoming an effective concentration for AP increase at a small infusion rate, as AP started to decrease at the scheduled end time irrespective of infusion rate.

Oscillations in AP are shown in the right panel of Fig. 6. The frequency of this oscillation correlated with the infusion rate. One cycle of AP oscillations occurred at 9.10 min for an infusion rate of 10 μl/h, at 4.55 min for 20 μl/h, and at 3.03 min for 30 μl/h. It took 36.40 min for one rotation of the cam at an infusion rate of 10 μl/h, 18.20 min at 20 μl/h, and 12.12 min at 30 μl/h. Therefore, one cycle of the AP oscillation corresponded with one quarter-rotation of the cam. In each quarter-rotation, a single cam projection sequentially pushes up each of the seven finger pins and compresses the tube. At the end of each quarter-rotation, the seventh finger pin suddenly retracts from the tube. This action generates negative pressure in the tube and backflow is induced. During this period, the solution is pulled back and is not infused into the animal. As the half-life of angiotensin II is 15 s (Al-Merani et al., 1978), AP starts to decrease soon after infusion ends. Once a single cam projection starts to push up the first finger pin again, positive pressure is induced and infusion starts. Accordingly, the oscillation characteristic is due to the property of the "Rotary Finger Method™".

In the present study, we have demonstrated the precision of programmed infusion rate and work time of the iPRECIO™ using an *in vitro* experiment, and we demonstrated the utility of the iPRECIO™ for *in vivo* experiments. Although the iPRECIO™ is a new type of infusion pump, the iPRECIO™ may have relative disadvantages compared to the implantable miniature pump. For most serious disadvantage is a weight of device. The mass of iPRECIO™ unit is 10 g, which is available for rats but too heavy for mice. On the other hand, osmotic pumps have a variety of lineup, consisted of 0.4, 1.1, and 5.1 g of weight, and are implantable for

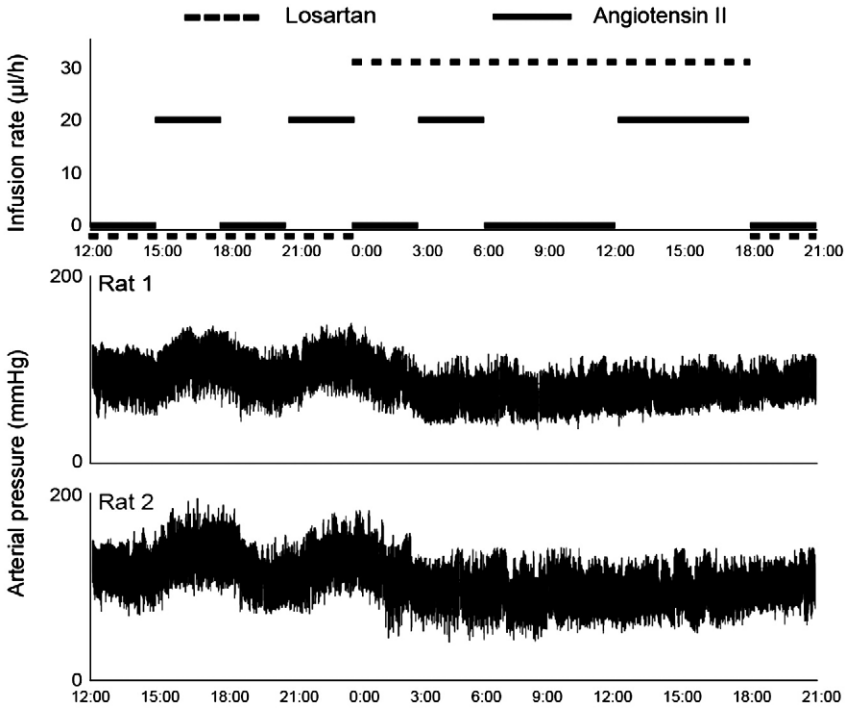

Fig. 9. Changes in AP in Experiment 3. Horizontal solid line indicates the period of angiotensin II (50 μg/ml) infusion; horizontal broken line indicates the period of losartan (50 mg/ml) infusion.

mice. However, the programmable and refillable features are notable, and thus the iPRECIO™ is suitable for use in a variety of experiments.

Acknowledgements

This study was supported by a Grant-in Aid for Scientific Research awarded by the Japanese Society for the Promotion of Science.

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