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Reduction in Animal Numbers by Long-term Implantation of Intravenous and Intra-arterial Catheters in Thyroparathyroidectomized Rats

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The thyroparathyroidectomized (TPTx) rat has been extensively used to study parathyroid hormone (PTH)-mediated bone resorption by measuring systemic Ca^{2+} concentrations. Animals have been traditionally used acutely; that is, they are often infused immediately after surgery and are sacrificed after a single use. To perform multiple experiments using a single group of animals we developed a system of long-term implanted intravenous/arterial catheters.Using calcitonin (CT) as a positive control, we successfully completed 12 separate controlled subexperiments documenting significant reductions in PTH-induced hypercalcemia in rats of the CT group. We then successfully completed two separate TPTx subexperiments, using a 3 × 3 Latin square experimental design. In both subexperiments, CT significantly inhibited the increase of blood Ca^{2+} concentration resulting from continuous PTH infusion. Our results indicate that, by combining the long-term use of catheters with the Latin square design, we can successfully reduce the number of animals used, increase the number of compounds screened, and improve the quality of the data. Although results of this study confirmed the acceptability of multiple infusions in anti-resorptive studies, investigations into the applicability of this set up to other areas of study requiring infusions and frequent blood sample collections seem appropriate.

Parathyroid hormone (PTH) is produced by the parathyroid gland, and plays a major role in regulation of calcium homeostasis in most vertebrate species (1). It stimulates release of calcium and phosphate from bone and increases reabsorption of calcium from the kidney and absorption from the intestines, while it decreases reabsorption of phosphate. Removal of this gland in preclinical species allows evaluation to be carried out in vivo of the effects of PTH on a variety of systems.

Thyroparathyroidectomized (TPTx) rats have been used for decades to study the actions and effects of PTH (2-4). These rats have been used to investigate hyperthyroidism (5), hyperparathyroidism (6), dentigenesis (7), bone formation (8, 9), and bone resorption (10-20). In bone research, the TPTx rat model is traditionally used as an acute and medium throughput in vivo screen to evaluate activity of compounds that are thought to inhibit bone resorption. This model provides data in as little as five (13) or six hours (11, 12) by measuring the ionized calcium (Ca^{2+}) concentration in whole blood, which increases in response to administration of PTH (17). By the co-infusion of PTH and a test agent, the compound's anti-resorptive activity can be determined by the measured inhibition of the blood Ca^{2+} increase.

Control of alternate blood calcium compensatory mechanisms is critical to allow appropriate data interpretation. Although it is logical that the model requires parathyroidectomy, the removal of the thyroid gland plays an important role in experimental control. The absence of this gland eliminates the effect of endogenous calcitonin (CT, a natural anti-resorptive hormone) secreted in response to an increase of blood calcium concentration (21). Further, dietary calcium intake is mitigated by consumption of a low-calcium diet and deionized drinking water.

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Most of the work using TPTx rats involves acutely prepared animals. This limits the usefulness of the method in several ways. First, animals prepared in this fashion are typically restrained physically (11, 13) or even chemically (12, 14) throughout the experiment. This presents the investigator with the substantial challenge of accounting for possible physiologic changes due to these added experimental variables. For example, kidney function has been documented to deviate from baseline while a study animal is anesthetized with common agents like pentobarbitals or ether (22). Second, PTH is often delivered via osmotic pumps, which have to be surgically implanted for the long term, and can only deliver the hormone at a steady rate. This approach does not allow flow rate adjustments during the course of the study or later "cross-over" opportunities (10, 15, 18). Third, the animals in most of the bone resorption studies are sacrificed after a single use (12). This results in long surgical periods, as well as extraneous labor costs from having to maintain large numbers of animals. This approach also antagonizes current ethical directives that encourage reduction in animal use. These factors led us to develop a system that would allow repeated use of a set of animals in several experiments. **(2)**

Long-term continuous intravenous infusion and blood collection via catheter systems in unrestrained rats is well established in animal research (23-31). It has been reported that continuous intravenous infusion for up to 90 days is safe in the rat (24). Long-term blood withdrawal from catheters in the unrestrained rat has been shown to be successful up to two (29) or three (23) weeks after implantation. In the study reported here, we describe the use of long-term implanted intravenous and intraarterial catheters, which allowed the reuse of test animals. We evaluated the usefulness of this system for testing bone anti-resorptive agents by carrying out 12 consecutive subexperiments over a six-week period in a single group of animals. In ad-

dition, we have evaluated the ability of this system to support experiments involving a crossover design.

Materials and Methods

Surgical animal procedures (experiment 1). All procedures were performed in accordance with protocols approved by the SmithKline Beecham Institutional Animal Care and Use Committee, and met or exceeded the standards of the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) and all local and federal animal welfare regulations. Conventionally raised male Sprague Dawley rats (average weight, 350 g) were received, having been previously thyroparathyroidectomized, from Taconic Farms (Germantown, N.Y.). On arrival, the animals were pair housed under conventional conditions and had ad libitum access to water and pelleted rodent chow (Purina Rodent Chow, 5001, Purina Mills, Inc., St. Louis, Mo.). After the cannulation, the animals were singly housed and had ad libitum access to de-ionized water and a low-calcium diet (Purina, Low Calcium Purified Diet, 5855C; 0.02% Ca content, Purina Mills, Inc., St. Louis, Mo.). Animals were maintained at 20 to 25°C room temperature with a 12/12-h light/dark cycle. Animal health status was evaluated, using a system where sentinel animals were distributed evenly throughout the room in the bottom row of cages, below the study animals. These sentinels were tested every four to six weeks and found free of rat coronavirus/sialodacryoadenitis virus, Kilham rat virus, Toolan's H-1 virus, Sendai virus, *Mycoplasma pulmonis*, pneumonia virus of mice, and parasites (including pinworms and mites).

One week after arrival, blood Ca^{2+} concentration was determined, using a Ca^{2+}/pH Analyzer (Model 634, Chiron Diagnostics, Norwood, Mass.), to ensure complete thyroparathyroidectomy. Only animals with blood Ca^{2+} values < 0.90 m*M* were used in the experiments. The cannulation technique of the femoral vein described by Cave and co-workers (24) was followed, with the following modifications. The animals were anesthetized by use of a 2.0 to 3.5% isoflurane/O_2 mixture, and a venous cannula (Micro-Renathane 0.040-in OD × 0.025-in ID, obtained from Braintree Scientific Inc, Braintree, Mass.) was inserted a distance of 5.5 cm. The same procedure was used for the cannulation of the femoral artery, where polyethylene tubing (Intramedic PE-50, 0.038-in OD × 0.023-in ID, Becton Dickinson & Co. Sparks, Md.) was inserted to 4.0 cm. After completion of the cannulation, both catheters were tied together with a silk suture, a subcutaneous pocket was created on the medial aspect of the thigh where both catheters were placed, and were secured to the thigh muscles, using silk suture (4-0, with an FS-2 cutting needle).

A small incision was made in the scapular region. Both catheters were fed through a stainless steel trocar (6 in × 14 gauge) from the inguinal area and exteriorized at the nape of the neck. The wounds were closed, using nylon suture (4-0, with an FS-2 cutting needle). Antibiotic ointment (Bactroban, mupirocin 2%, SmithKline Beecham, Philadelphia, Pa.) was applied to the exteriorization site to minimize microbial growth. Animals received Banamine (1.0 mg/kg of body weight, s.c., Flunixin Meglumine, Schering-Plough Animal Health, Union, N.J.) immediately after surgery as an analgesic (32). The animal was then fitted with a jacket of appropriate size. The tubing was protected by an 18-in-long stainless steel spring attached to 22-gauge single channel swivel mounted on a standard wire housing rack (the jacket/tether system was purchased from Lomir Biomedical Inc., Malone, N.Y.). Venous patency was maintained by a continuous infusion of Ringer's lactate (Ringer's Injection, USP; 2B2303, Baxter HealthCare Corporation, Deerfield, Ill.) at a rate of 0.90 ml/h delivered by a syringe pump (Model PHD2000 and Model 22, Harvard Apparatus Syringe Pump, South Natick, Ma.).

Arterial patency was maintained by use of a sodium heparin solution (500 I.U./ml in 50% dextrose), with an injected lock that was changed daily. Both systems were closed to air by use of injection caps. However since the injection cap system does not provide an absolute airtight environment, the non-infused arterial line was clamped with a small binder clip to prevent the flow of blood back into the catheter. The venous line did not require such clip because the force of infusion and flow characteristics of the vein prevent backflow. Because of the absence of the thyroid gland, thyroxine (2 μg/rat, 3 times/wk; L-thyroxine obtained from Sigma Chemical Co., St, Louis, Mo.) was administered subcutaneously to maintain basal metabolic rate. All animals received daily environmental enrichment (peanuts, paper towels, and nestlets).

Surgical and animal procedures (experiment 2). The aforedescribed procedure was used in the second experiment with the exception that the calcium-deficient diet was obtained elsewhere (AIN-76A, 0.01% Ca content, Ziegler Brothers Inc., Gardeners, Pa.). Average body weight at the time of surgery was 415 g. Venous catheter patency was maintained by continuous infusion of Lactated Ringer's Injection USP (2B2324, Baxter HealthCare Corporation.) at a rate of 1 ml/h for three days immediately after surgery, then with Ringer's Injection USP (2B2303, Baxter HealthCare Corporation) at an infusion rate of 0.90 ml/h. Animals were housed in standard polycarbonate cages, which required the tether to be 24 in long. The tether entered the cage through a narrow slit made in the front and extending approximately three quarters of the height. A thyroxine-containing pellet (L-thyroxine, T4; 0.25mg, 60-day release, ST-131, Innovative Research of America, Sarasota, Fla.) was implanted subcutaneously at the time of cannulation.

Experimental procedures (experiment 1; parallel group experimental design). Prior to each infusion, a hypocalcemic baseline was established to serve as a reference point against changes in blood calcium concentration at later time points. This was achieved by: permanent transfer to deionized drinking water, permanent transfer to the low-calcium diet, and allowing no less than 72 h between separate PTH infusions to allow blood calcium values to return to the natural baseline.

The animals were sorted into vehicle group (saline; n = 9), PTH group (human PTH 1-34, 1.25 μg/kg/h, Bachem Bioscience, King of Prussia, Pa.), and PTH+CT group (PTH as described previously, salmon thyrocalcitonin, 4.0 μg/kg/h [Sigma Chemical Co.]). Infusion rates were calculated on the basis of body weight, and concentrations were adjusted so that flow rates were dependent on an average infusion of 1 ml/h. Animals were removed from the study when the arterial line lost its patency (see Table 1 for group sizes). All subexperiments lasted exactly six hours, with blood withdrawals at 0, 3, and 6 h (0.3 ml/sample). Immediately following a collection time point, whole blood was analyzed for Ca^{2+} concentration (Ca^{2+} values were adjusted to pH of 7.4).

After completion of 12 subexperiments (or when removed from the study), all animals were euthanized by use of CO_2 asphyxiation and necropsy was performed to assess catheter placement and any gross abnormalities.

Table 1. Design of experiment 1 (parallel groups)

	No. of animals in each group				
Subexperiment	Vehicle	PTH	PTH + CT	Total	Body weight (g) ± SEM
1	9	10	10	29	351 ± 3
2	8	10	10	28	352 ± 3
3	8	10	10	28	357 ± 4
4	8	9	9	26	359 ± 4
5	8	8	9	25	362 ± 5
6	8	8	9	25	362 ± 5
7	8	8	8	24	374 ± 7
8	7	8	8	23	375 ± 7
9	6	8	8	22	380 ± 7
10	6	8	8	22	380 ± 7
11	5	8	8	21	388 ± 8
12	3	8	8	19	382 ± 9

PTH = parathyroid hormone.
CT = calcitonin.

Experimental procedures (experiment 2; Latin square crossover study design). A hypocalcemic baseline was established as described previously. In addition, the animals were transferred from the Ringer's infusion to saline, approximately 24 h prior to the start of each PTH infusion. The PTH was delivered at a rate of 1.25 μg/kg/h and CT was given at the rate of 1.0 μg/kg/h. The vehicle consisted of 0.1% bovine serum albumin (BSA, Sigma Chemical Co.) in saline. A 3 × 3 Latin square crossover design was used (Table 2). In this design, each animal was exposed to each treatment once and each treatment was tested on each experimental day. Three animals were randomly assigned to one of three groups (vehicle, PTH, PTH+CT) on the morning of day 1, and the design was followed three times over a period of a week and a half. This subexperiment was repeated twice, using the same animals. After completion of both subexperiments, the animals were euthanized by CO2 asphyxiation and necropsy was performed to determine catheter placement and any gross abnormalities.

Data analysis. All data are expressed as mean ± SEM. One-way analysis of variance (ANOVA) with Tukey's test was used to detect significant differences between treatment groups in the first experiment. Three-way ANOVA for Latin square, multiple comparisons, followed by pairwise adjustments was used to detect differences between treatment groups in the second experiment. Probabilities were calculated on the basis of net changes in circulating Ca^{2+} values. Significance was denoted by $P \leq 0.05$. Data were analyzed, using Microsoft Excel (Microsoft, Santa Rosa, Calif.), STATISTICA (Statsoft, Tulsa, Okla.), and SAS (SAS Institute, Cary, N.C.).

Results

Experiment 1 (parallel group design). Twelve separate TPTx rat subexperiments were completed in six weeks, using the same group of animals. The minimal number of animals needed to detect significant differences between treatment groups was three for the vehicle group (negative control), eight for the PTH-only group (positive control), and eight for the PTH+CT (these numbers were obtained from sample size calculations using data from our previous work with this model). Experiment 1 began with 29 animals, and the subexperiments continued for six weeks until the number of animals on study reached the lower statistically allowable limit of 19 (Table 1). Animals were eliminated from the experiments when an arterial cannula (blood withdrawal) became occluded and non-functional. Venous cannulas in all rats remained patent for infusion for the duration of the study.

Table 2. Design of experiment 2 (Latin square)

Animal No.[a]	1	2	3
Day No.	Group assignments (experiment 1)		
1	PTH+CT	PTH	Vehicle
2	Vehicle	PTH+CT	PTH
3	PTH	Vehicle	PTH+CT
Day No.	Group assignments (experiment 2)		
1	Vehicle	PTH+CT	PTH
2	PTH	Vehicle	PTH+CT
3	PTH+CT	PTH	Vehicle

[a]Each rat was exposed to each treatment on three separate days in both experiments.
The same animals were used in both experiments.

The results from all experiments are presented in Table 3. There was an overall trend toward an increase in mean blood Ca^{2+} concentration before the PTH infusion (0.75 m*M* in the first subexperiment to 0.86 m*M* in the last). Calcitonin completely inhibited the calcemic response to PTH in each subexperiment. The overall calcemic response to the six-hour PTH infusion indicated a trend toward a decrease as the series of subexperiments progressed. This was due to an increase in variability of calcemic response in the PTH-treated group (Fig. 1), starting with subexperiment 6. In subexperiments 10-12, the blood Ca^{2+} concentration was not significantly different from that of the vehicle group (*P*-values slightly higher than 0.05, see Table 3). Some of the animals did not respond at all to the PTH infusion in the last three subexperiments. These data suggest that there may be desensitization to repeated PTH exposure, although this phenomenon did not occur in all animals. Taken together, these data indicate that we can confidently use rats cannulated in this manner for a period of four weeks (eight subexperiments). Statistical power calculations are shown in Table 3. With the parallel group design, on average, we can detect a difference of approximately 0.40 m*M* with a power level of 0.95 (0.05 probability of type-II error).

Mean body weight loss of 9 g was observed immediately after the surgery. Subsequently, constant weight gain was observed (Table 1). All animals appeared to be in good condition for the duration of the study. At necropsy, the tip of the venous cannula was found to be placed incorrectly (short of the desired 5.5 cm distance) in nine of 29 rats (this did not have any effect on catheter patency). The arterial cannula was placed correctly (to depth of 4 cm) in 16 rats. In the other 13 rats, the catheter was inserted only partially (to a distance of ~ 2cm). In five of these rats, the catheter remained patent for the duration of the study (48 days after surgery). In the remaining eight rats, the catheter became occluded as early as nine days after surgery. Only one rat with a correctly placed arterial catheter was removed from the study before its completion (day 30 after surgery). One rat chewed a hole in the arterial line under the jacket and was euthanized on day three (seven days after cannulation). Necropsy also revealed presence of a fibrous mass around the catheter in the vena cava in six animals. Ten rats had somewhat pale kidneys, and one rat had a necrotic left kidney, with a normal right kidney. Three rats were found to have enlarged prostate. These findings were unrelated to the apparent loss of the response to PTH infusion.

Experiment 2 (Latin square design). Having established that we could re-use cannulated rats for at least four weeks, we tested whether this system would support a Latin square experimental design. We conducted two separate subexperiments, using a 3 × 3 Latin square in three rats. In this experimental

Table 3. Summary of all subexperiments in experiment 1 (parallel group design)

Subexperiment	All groups 0 h	Vehicle group 0 h	Vehicle group 6 h	PTH group 0 h	PTH group 6 h	PTH + CT group 0 h	PTH + CT group 6 h	Minimal difference[a]
1	0.75 ± 0.04	0.74 ± 0.06	0.68 ± 0.06	0.79 ± 0.06	1.22 ± 0.07‡	0.73 ± 0.03	0.64 ± 0.02¶	0.34
2	0.75 ± 0.04	0.76 ± 0.09	0.77 ± 0.08	0.76 ± 0.07	1.15 ± 0.11*	0.74 ± 0.03	0.69 ± 0.03¶	0.52
3	0.84 ± 0.03	0.82 ± 0.06	0.80 ± 0.08	0.84 ± 0.03	1.28 ± 0.05‡	0.84 ± 0.06	0.80 ± 0.03¶	0.25
4	0.83 ± 0.03	0.83 ± 0.04	0.80 ± 0.06	0.81 ± 0.07	1.26 ± 0.12†	0.83 ± 0.06	0.76 ± 0.04¶	0.57
5	0.84 ± 0.03	0.86 ± 0.09	0.86 ± 0.08	0.84 ± 0.04	1.29 ± 0.06‡	0.83 ± 0.03	0.77 ± 0.02¶	0.31
6	0.84 ± 0.03	0.84 ± 0.04	0.86 ± 0.05	0.85 ± 0.09	1.15 ± 0.12*	0.83 ± 0.04	0.74 ± 0.03§	0.57
7	0.84 ± 0.04	0.83 ± 0.05	0.86 ± 0.04	0.84 ± 0.07	1.27 ± 0.03‡	0.84 ± 0.08	0.80 ± 0.06¶	0.17
8	0.89 ± 0.04	0.89 ± 0.05	0.81 ± 0.05	0.91 ± 0.09	1.29 ± 0.09†	0.88 ± 0.06	0.78 ± 0.05¶	0.43
9	0.81 ± 0.03	0.81 ± 0.10	0.84 ± 0.11	0.81 ± 0.05	1.18 ± 0.06†	0.81 ± 0.02	0.79 ± 0.02¶	0.29
10	0.82 ± 0.03	0.82 ± 0.06	0.84 ± 0.06	0.81 ± 0.07	1.07 ± 0.09 (P=0.063)	0.82 ± 0.04	0.76 ± 0.03¶	0.43
11	0.90 ± 0.03	0.89 ± 0.03	0.89 ± 0.05	0.89 ± 0.05	1.16 ± 0.09 (P=0.056)	0.91 ± 0.07	0.87 ± 0.06¶	0.50
12	0.86 ± 0.03	0.87 ± 0.05	0.75 ± 0.09	0.85 ± 0.04	1.11 ± 0.09 (P=0.052)	0.86 ± 0.07	0.81 ± 0.06§	0.45

All values represent whole blood Ca^{2+} concentration, and are expressed as mean +/– SEM (mM).
*, †, ‡ = P < 0.05, 0.01, 0.001, respectively, vs. vehicle group.
§, ¶ = P < 0.01, 0.001, respectively, vs. PTH group.
[a]Minimal difference in whole blood Ca^{2+} between PTH and PTH+CT groups that is necessary to detect statistically significant inhibition of the calcemic response at statistical power of 0.95 (type-II error probability of 0.05).

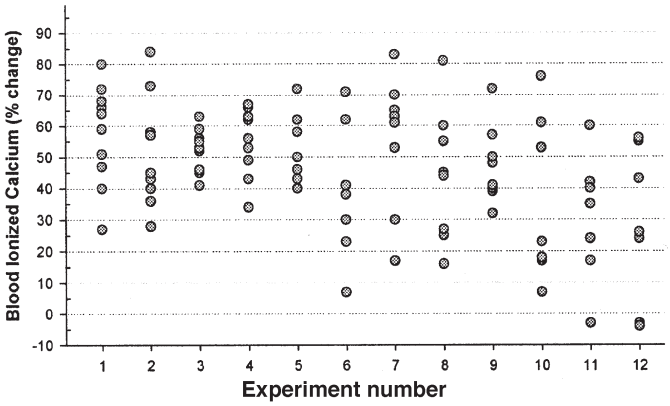

Figure 1. Summary of responses to parathyroid hormone (PTH) in experiment 1 (parallel group design) through 12 separate subexperiments. Each circle represents increase in whole blood Ca^{2+} concentration caused by a six-hour infusion of PTH (data are expressed as percentage change from baseline). Variability increased somewhat, starting with subexperiment 6. At that point, some of the animals started to loose the ability to respond to PTH.

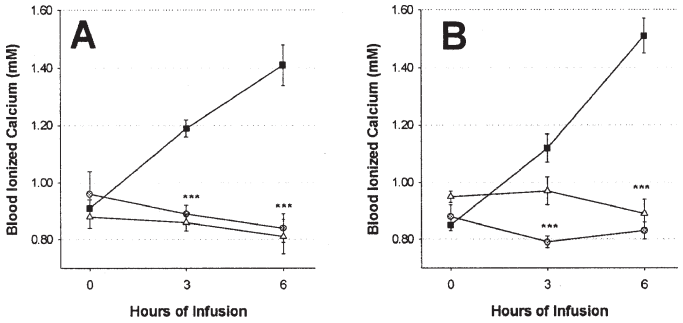

Figure 2. Whole blood Ca^{2+} concentrations in experiment 2 (Latin square design), from both subexperiments, 1 (panel A) and 2 (panel B). Each point represents mean (± SEM) data from the same three animals infused with vehicle (clear triangles), PTH (black squares) or PTH plus calcitonin (CT) (gray diamonds). Each rat received all three treatments on different days in such a way that two treatments were not tested on the same day. Asterisks (***) indicate significant ($P < 0.001$) difference versus PTH group, no difference vs vehicle group.

design, each subject receives all of the treatments, each acts as its own control, and treatments can be compared within subjects. This reduces between-animal variability estimates, but needs a "wash-out" period to allow the return to baseline, so it is best suited to acute studies, such as those with TPTx rats.

The results from both subexperiments are shown in Fig. 2. In both instances, a significant increase in blood Ca^{2+} concentration due to the six-hour PTH infusion was observed. This PTH response was completely inhibited by co-infusion with CT. Animals treated with the vehicle did not have significant change in blood Ca^{2+} values in either subexperiment. In experiment 1, the minimal detectable difference in concentration of whole blood Ca^{2+} concentration at hour six was 0.075 m*M* at statistical power of 0.95. Due to higher variance in experiment 2 (SD of 0.11, as opposed to 0.03 in experiment 1) the minimal detectable difference increased to 0.281 m*M* at power of 0.95. In each instance, the detectable differences were in the same range or lower than those observed in the parallel group design. That is, the Latin square design had equivalent or greater sensitivity. The statistical power achieved here required only three animals, compared with the 19 to 29 used in the parallel group method.

All animals appeared to be healthy for the duration of both experiments. Necropsy did not reveal any abnormalities, and all catheters were placed correctly. Only two experiments using this design were run because one animal destroyed its arterial cannula and had to be euthanized.

Discussion

We have developed a system involving long-term implantation of intravenous and intra-arterial catheters that allows us to complete several experiments in TPTx rats, using the same group of animals. The data presented here indicates that our experimental design made it possible to obtain consistent and reproducible data for at least four weeks while using each animal twice a week. In all of the described experiments, PTH, delivered as a six-hour intravenous infusion at a rate of 1.25 μg/kg/h, increased whole blood Ca^{2+} concentration sufficiently to allow measurement of the inhibition of this response by use of anti-resorptive treatment. Parathyroid hormone delivered at this rate has minimal effects on the renal handling of calcium, so the increase in blood Ca^{2+} concentration is due to bone resorption not calcium conservation (2). Calcitonin, a clinically approved anti-resorptive, was used in our studies to validate the model design. Calcitonin completely inhibited the calcemic response in all experiments. In the first experiment, CT was delivered at a rate of 4.0 μg/kg/h (co-infused with PTH). Later, through dose-ranging

subexperiments, we found out that this dosage could be lowered, and in the second experiment, CT was administered at the rate of 1.0 μg/kg/h, which was equally effective.

In addition to achieving a great increase in the number of tests to be carried out over a short period, we also found it possible to run cross-over experiments, including the Latin square design. Using this experimental design, statistical power increased while the required number of animals was reduced. In this design, each subject (animal) is exposed to each treatment over the course of several test days and, within each day, all of the treatments are represented (33). Each animal acts as its own control so treatments can be compared within subjects. The only drawback to this design is that the main measure has to return to baseline values before each subsequent experiment, since the assumption has to made that the subjects are identical at that point.

In the parallel group design, using 19 to 29 animals in three groups, with statistical confidence of 95%, the detectable difference was about 0.4 m*M*, whereas in the Latin square design, using three animals in three groups, we could detect a difference of 0.2 m*M*. By using six animals (i.e., creating two replicates of a 3 × 3 design) the detectable difference with 95% power would have been decreased even further to 0.075 to 0.1 m*M*. It is clear from our results that this system can support crossover designs that reduce animal numbers and increase statistical power.

Continuous infusion and blood collection in unrestrained rats is well established in animal research (23-29, 31). Continuous intravenous infusion with physiologic saline at rate of 1 ml/h for up to 90 days was observed not to cause adverse effects, compared with results in non-infused animals (24, 27). Long-term blood withdrawal from catheters in the unrestrained rat has been documented to be successful for up to two (29) or three (23) weeks after implantation. We applied and modified some of those techniques in our experiments by use of a commercially available tethering/swivel device. Using a stainless steel spring tether, the catheter was protected while patency was maintained by continuous infusion or by locking the catheter with a heparin/dextrose mix.

For our infusion/collection method to be successful, longevity of the rats has to be ensured. To avoid losing animals before the study was completed, we found that the arterial catheter tip placement was one of the more important factors. The femoral artery is reported to be the best site for repeated blood withdrawals (29); however, the surgery (especially when using PE-50 tubing, one that we found to be most appropriate in our studies) requires some skill and practice. We were forced to remove a number of animals from the first experiment because the arterial cannula stopped functioning. At necropsy, we found that, in most instances, the cannula was not inserted sufficiently deep inside the vessel (< 4 cm) or was not secured properly with suture material. We found that if the ligature around the arterial cannula was not sufficiently tight, it could be slowly pulled out (there is a constant slight pull on this cannula since it is not attached to the swivel and hangs free). These problems can be avoided by inserting the cannula to the depth of 4 cm, tying at least three ligatures around it, and by anchoring it in two places to the thigh muscle. This still does not guarantee that blood withdrawal may be continued indefinitely, but at least some of the problems can be avoided.

To discourage the animals from disturbing the jacket (or harness) and tether system, the rats were maintained on an environmental enrichment program. This consisted of either nestlets or a paper towel (given to each animal daily), or various treats (peanuts, dried bananas, dried apples, grapes, rawhide chews). Certain types of treats or chews, appropriate for rodents, could not be used while conducting studies using the TPTx rat because of the suspect calcium content. Grapes, raisins, and rawhide chews could only be given to the animals between experiments, and had to be removed 48 h in advance of PTH infusion to ensure that the lowest possible hypocalcemic baseline was achieved.

After the fourth week of the six-week subexperiment series (experiment 1), some animals lost the ability to respond to PTH. We are uncertain of the cause, since not all animals behaved in this manner. One possibility might be that the PTH receptors become desensitized to PTH. However, since this animal model is not used long term, there are no reports about this in literature. The calcemic response to continuous infusion of PTH decreases with time in parathyroid-intact animals due to the action of endogenous CT (4, 21). However, this cannot explain why this response is diminished in TPTx animals, which have no endogenous CT, unless the TPTx was not entirely successful and some remaining thyroid tissue regenerated to the point of being effective as an anti-resorptive. More studies are needed to answer this question. Because of this problem, we routinely do not use TPTx animals for more than four weeks. Using this experimental design, it is still possible to run up to eight subexperiments since the calcemic response appears unaffected during this time frame. The small increase in the hypocalcemic baseline that we saw in experiment 1 (Table 3) did not appear to interfere with the action of PTH.

The infusion and blood collection system described here could be applied to studies other than anti-resorptive experiments with the TPTx rat. Any long-term study that requires infusion and frequent blood collection, or a short-term study that does not require long recovery time between separate treatments could be conducted in this manner. The repeated use of cannulated animals in multiple studies greatly reduces the overall number of animals needed. This can also accommodate use of a Latin square experimental design, which not only reduces the numbers even further, but also reduces the supply requirements for test compounds. In addition, using our method, the statistical power increases, compared with that on studies run by use of a parallel group design (the detectable difference can be decreased by as much as a factor of five).

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