MEASUREMENT OF PLASMA CALCITONIN IN VIVO IN THE PIG USING A CHRONICALLY-IMPLANTED CATHETER FOR INFUSION AND BLOOD SAMPLING

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Summary

A method used to monitor circulating levels of calcitonin (CT) in the pig by means of a chronically-implanted, indwelling jugular catheter was described. This catheterization method facilitated repeated infusion and blood sampling in conscious and unrestrained animals for periods of several months. Since animals with jugular catheters in place may be used repeatedly and since the CT level in the jugular blood was observed to be several-fold greater than in the peripheral circulation, this catheterization method provides definite advantages over other sampling techniques which have previously been used to evaluate CT secretion in the pig.

Introduction

Calcitonin is the hypocalcemic hormone which is synthesized and secreted by the parafollicular cells of the thyroid. Accurate physiologic studies of this hormone often require intermittent infusion and/or blood sampling in conscious, unrestrained animals. The difficulties normally encountered with these procedures are compounded in the pig because of the paucity and extreme fragility of superficial vessels. Jugular catheterization of the pig has been described previously for the purpose of either infusion or blood sampling but not for both simultaneously (Zimmerman et al., 1973; Shearer and Neal, 1972; Christison and Curtin, 1969; Ralston, Kleber and Smith, 1949). The present study employed a modification and extension of the above techniques and was used for concomitant infusion and blood sampling in conscious and anesthetized pigs for periods of up to 2 months. This report describes the advantages and flexibility of this method for the in vivo study of circulating levels of CT in the pig.

Materials and Methods

Young male Yorkshire pigs were anesthetized with an injection of sodium pentobarbital (40 mg/kg, im), and the entire neck and throat areas were shaved and prepared for aseptic surgery. A vertical incision 8 to 9 cm in length was made in the left throat directly below and behind the mandible (figure 1A), and a second vertical incision, approximately 2 cm long, was placed immediately behind the left ear (figure 1B).

The sterno-hyoideus muscle was retracted and internal jugular isolated by blunt dissection. A polyethylene catheter with stylet (Brad #1936-R, Murry Hill, N.J.) was passed subcutaneously, with the aid of 8-inch hemostatic forceps, from the small incision behind the ear to the jugular vein in the neck. The catheter was then tunneled subcutaneously to a small subcutaneous pocket behind the ear. A 3-way stopcock was attached to the catheter and the entire site was closed by sutures. The catheter was then tunneled subcutaneously to a small subcutaneous pocket behind the ear. A 3-way stopcock was attached to the catheter and the entire site was closed by sutures.

Figure 1. Anesthetized pig shortly after catheter implantation. The surgical incisions are indicated by arrows A and B.

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2 Department of Pharmacology.
and brought out in the anterior part of the larger incision, near the jugular. The isolated vessel was clamped shut posteriorly and securely ligated anteriorly. A small opening was then made in the center of the exposed jugular. The catheter was flushed with heparinized saline, inserted posteriorly into the vessel, and passed into the area of the thyroid venous drainage. Once in place, the catheter was ligated to the vessel and anchored to the surrounding musculature. A 5 cm loop of catheter was included to allow for head movement and animal growth. Both incisions were then closed and the wounds dressed with 3 Antibiotic Ointment (Day Baldwin, Inc.; Hillside, N.J.). The dressing and catheter were held in place with several layers of adhesive tape passed around the circumference of the neck. Animals were treated prophylactically with penicillin G (250,000 units, ira) and the dressing was changed biweekly.

The patency of the catheter was maintained by heparinizing all experimental infusates (100 units/ml) and by replacing the stylet between experiments. The use of a stylet several centimeters longer than the catheter was found to prevent clot formation at the indwelling tip of the catheter.

Infusion and blood sampling with a single indwelling catheter was accomplished by connecting the infusion line and external catheter with a three-way stopcock as shown in figure 2. Intermittent sampling during an infusion was performed by turning the stopcock to the sampling line, flushing the catheter with several milliliters of saline, and aspirating the blood sample. The first 2 ml of each sample were collected separately and discarded to prevent sample contamination by infusate. The stopcock was then returned to the infusion line. Sampling in this manner caused only a 45- to 60-sec. interruption of the experimental infusion.

In the feeding study the animals were fasted for 24 hr. and then received either a low calcium diet containing 0.02% calcium (General Biochemicals) or a normal diet (low calcium diet supplemented with 1.5% calcium as calcium carbonate) and were allowed to feed ad libitum for 45 minutes. The plasma CT levels during feeding and postprandially were compared to CT levels measured at 10-min. intervals during the 30-min. period immediately prior to the onset of feeding. In the calcium infusion study the animals were fasted overnight and anesthetized with pentobarbital before each experiment. After a 30-min. control period, a calcium solution was infused via the indwelling jugular catheter at a rate of 1 ml/min. during the 30 min. infusion period using a Harvard variable speed infusion pump (model 600-955 UDC). Calcium infusates were prepared with CaCl₂ · 2 H₂O in distilled water. The statistical comparison between plasma CT levels at different time periods was conducted by means of the t test for correlated data.

Plasma CT was measured by a specific and sensitive radioimmunoassay for porcine CT which previously has been described in detail (Pento, Glick and Kagan, 1973a).

Results and Discussion

Infusion and plasma sampling, as described above, were utilized successfully in 10 pigs ranging in weight from 5 to 40 kilograms. The use of this sampling method permitted CT measurement during normal feeding and postprandially in conscious animals confined to their cages (Pento, 1971).

The results of a feeding study are presented in figure 3. The animals consumed from 0.45 to 0.7 kg of the diet during the feeding period and appeared to consume both diets equally well. The calcium supplemented diet produced a significant rise in plasma CT from 30 to 90 min. after the onset of feeding (P < .05). However, after the calcium deficient diet, CT levels were reduced slightly (not significant). These data suggest that the absorption of dietary calcium may stimulate CT secretion directly or indirectly by the release of a gastrointestinal hormone such as glucagon, gastrin or pancreozymin which have previously been shown to influence CT secretion (Care et al., 1971; Cooper et al., 1971b). The CT released into the circulation could help maintain calcium homeostasis postprandially during the rapid influx of dietary calcium.
CHRONIC JUGULAR CATHETERIZATION IN THE PIG

Figure 3. Influence of dietary calcium on CT secretion. Each point represents the mean value for CT in the plasma from five pigs at the times indicated ± SE. Plasma CT levels during the feeding and postprandial periods were statistically compared to the mean CT level measured during the 30-min. period immediately prior to feeding (indicated by the horizontal cross-hatched bar).

The results of a calcium infusion study are summarized in figure 4. The 10 mg/kg calcium infusion produced a significant (P < .01) threefold rise in plasma CT (figure 4A) while the 15 mg/kg infusion caused a much greater fivefold increase in the circulating level of this hormone (figure 4B) (P < .01). This study illustrates the effectiveness of this sampling method in the measurement of plasma CT during infusion experiments.

Since catheterization was employed to measure the secretion of porcine CT in vivo, the catheter was placed near the thyroid venous drainage, thus permitting the detection of greater than normal peripheral levels of the hormone. This factor becomes very important when measuring a polypeptide such as CT which is present at subnanogram concentrations in the peripheral circulation of the pig (Lequin et al., 1969; Colt et al., 1971). Sampling with this method, the mean plasma CT level measured by radioimmunoassay in 182 samples from 10 pigs was approximately fourfold greater than CT levels reported by other investigators in peripheral plasma (see table 1). Conversely, the jugular plasma CT levels are much lower than CT levels previously reported in in situ experimentation (Cooper, Deftos and Potts, 1971a).

The present sampling method presents certain advantages over both the in situ and peripheral plasma sampling techniques. Since the jugular method allows the measurement of plasma CT levels which are greater than the levels found in the peripheral circulation, it is much easier to measure changes in CT secretion (both increased or decreased secretion) and to evaluate factors which may alter CT secretion.

Figure 4. The influence of a calcium infusion on CT secretion. Each point represents the mean value for CT in the plasma from five pigs at the times indicated ± SE. The mean plasma CT level during the 30-min. infusion period was statistically compared to the mean plasma CT level during the 30-min. preinfusion period.
<table>
<thead>
<tr>
<th>No.</th>
<th>Animal</th>
<th>Weight range (kg)</th>
<th>No. of samples</th>
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<tr>
<td>1</td>
<td>BK</td>
<td>28.2 – 33.8</td>
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<td>1.12 ± 0.043</td>
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<tr>
<td>2</td>
<td>MX</td>
<td>32.0 – 39.6</td>
<td>20</td>
<td>1.15 ± 0.048</td>
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<tr>
<td>3</td>
<td>MD</td>
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<td>17</td>
<td>1.01 ± 0.043</td>
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<tr>
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<td>17.3 – 27.4</td>
<td>6</td>
<td>1.32 ± 0.044</td>
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<tr>
<td>5</td>
<td>P1</td>
<td>14.4 – 18.0</td>
<td>26</td>
<td>1.34 ± 0.034</td>
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<tr>
<td>6</td>
<td>P2</td>
<td>12.5 – 17.1</td>
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<tr>
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<td>BG</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>182</td>
<td>1.19d</td>
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</table>

*All samples were drawn by means of the jugular catheter.

Animal weight range during the period that the animal was being used for experimentation. This period varied from 2 to 8 weeks in various animals.

Total number of samples measured.

Grand mean of the basal plasma CT from all 10 animals studied.

(Pento et al., 1973b). Further, jugular catheterization provides a convenient portal which may be used repeatedly for chronic experimentation rather than in a single acute experiment. In addition, infusion and sampling can be conducted in conscious and unrestrained animals confined to their cages and during natural sleep.

**Literature Cited**


