EFFECTS OF ELCATONIN ON PLASMA AND URINE ELECTROLYTES IN INFANT BEAGLES

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Abstract—The effects of elcatonin (ECT) on the renal hemodynamics and electrolyte levels in plasma and urine were investigated in anesthetized infant beagles (3 months old) and adult beagles (9–10 months old). Intravenous injections of 50 MRC U/kg ECT did not alter the systemic blood pressure, renal blood flow, glomerular filtration rate, and urine flow in intact puppies as well as in puppies and adult dogs subjected to extirpation of the thyroid and parathyroid glands (TPTX). One hundred and twenty min after ECT injection, the plasma calcium concentration decreased by 1.06±0.15 mEq/l in intact puppies and 0.94±0.10 mEq/l in TPTX puppies, but decreased only by 0.38±0.22 mEq/l in adult TPTX dogs. The urinary calcium excretion decreased in all the puppies. The plasma phosphorus concentration decreased by 1.02±0.10 mg/dl, and the phosphorus excretion rate increased 63.6±26.4% in intact puppies; whereas the plasma phosphorus and phosphorus excretion were not altered in TPTX puppies. Diuresis and natriuresis were not obtained. These data suggest that the acute hypocalcemic effect of ECT is dependent on age and is not associated with its action on kidneys.

Calcitonin, a hormone secreted from the C cell of thyroid glands, lowers the plasma calcium concentration by a direct inhibition of bone resorption. The hypocalcemic effect is also observed in nephrectomized, gastro-enterotomized or parathyroidectomized rats (1). Calcitonin increases the urinary excretion of inorganic phosphate, sodium and potassium in mammals including humans (2). The effect of calcitonin varies in different species, strains, ages, experimental conditions etc; therefore, its effects have to be analyzed under controlled conditions.

The purposes of the present study were to clarify the renal and hypocalcemic actions of a high dose of synthesized eel calcitonin analogue: elecatonin (ECT) in beagles, to determine the involvement of the parathyroid glands in the action of calcitonin, and to compare the action of calcitonin in puppies and adult dogs. ECT is chemically stable and possesses a hypocalcemic potency similar to that of natural eel and salmon calcitonin (3). The amino acid sequence of ECT, [Asu1-17]-eel-calcitonin (4), is as follows: Asu-Ser-Asn-Leu-Ser-Thr-Asu-Val-Leu-Gly-Lys-Leu-Ser-Gln-Glu-Leu-His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asp-Val-Gly-Ala-Gly-Thr-Pro-NH2 (Asu: L-α-Aminosuberic acid). The specific activity is 5000 MRC U/mg.

MATERIALS AND METHODS

Studies were carried out on three groups of male beagles. Group 1 consisted of 5 intact puppies (100±6 days old) weighing
4.4±0.6 kg. Group 2 consisted of 6 thyroid-parathyroidectomized (TPTX) puppies (100±7 days old) weighing 5.0±0.6 kg, and Group 3 consisted of 4 TPTX adult dogs (9-10 months old) weighing 9.9±0.4 kg. Standard dog food was given until the day before the experiment and water was given ad libitum.

The puppies and adult dogs were anesthetized with pentobarbital Na (30 mg/kg, i.v.). Artificial ventilation was provided with an artificial respirator (Igarashi B-2). A polyethylene catheter was inserted into the abdominal aorta through the left femoral artery and placed close to the orifice of the left renal artery. Thus, the blood pressure measured through this catheter was considered to represent the renal arterial pressure. Then the thyroid and parathyroid glands were extirpated in 6 puppies (Group 2) and 4 adult dogs (Group 3). The left renal artery was exposed via a retroperitoneal flank incision, and all visible nerve fibers entering the renal hilum were sectioned. The left ureter was cannulated, and the urine flow was measured by timed collections. The renal blood flow (RBF) was measured by an electromagnetic flow meter (Nihon Kohden MF-26). The glomerular filtration rate (GFR) was measured by means of the exogenous creatinine clearance. The right brachial artery was cannulated for the collection of blood samples. The right brachial vein was cannulated, and an isotonic saline solution including creatinine (8.3 mg/ml) and pentobarbital Na (1 mg/ml) was infused at a constant rate of 0.1 ml/kg/min following the primary administration of creatinine (100 mg/kg). The infusion of this solution was maintained throughout the experiments. After the surgery was completed, the control animals (Group 1) were allowed to equilibrate for 30–40 min, and the TPTX animals for 120 min. After the equilibration period, urine was collected for 10–15 min three times. Arterial blood samples were also obtained at the mid-point of the period for urine collection. Mean values of parameters measured from these three samples of plasma and urine were taken as control values. After the period of control sampling (30–45 min), 50 MRC U/kg of elcatonin (ECT) was injected into the left forearm vein. Blood and urine were collected at 15, 30, 60, 90, and 120 min after the ECT injection.

Creatinine was assayed calorimetrically (5). Concentrations of calcium, sodium and potassium in plasma and urine were determined by an atomic absorption spectrometer (Varian Techtron, Model 1100). Phosphorus in plasma and urine were analysed by the method of Fiske and Subbarow (6).

RESULTS

Effect of ECT on renal hemodynamics: Injection of ECT (50 MRC U/kg, i.v.) did not alter the mean renal blood pressure in intact puppies (N=6), TPTX puppies (N=6) and TPTX adult dogs (N=4) in which absolute values of the blood pressure during a control period averaged 111±7.5 mmHg, 99.8±3.0 mmHg and 128±6.7 mmHg, respectively. Renal blood flow (58.8±8.2 ml/min in intact puppies, 68.9±6.6 ml/min in TPTX puppies and 87.7±21.6 ml/min in TPTX adult dogs) was not significantly influenced by the injection of ECT. Glomerular filtration rate during a control period averaged 12.2±2.7 ml/min, 14.5±1.5 ml/min, and 22.9±2.1 ml/min in intact puppies, TPTX puppies and TPTX adult dogs, respectively. The glomerular filtration rate was unaffected by the injection of ECT. Glomerular filtration rate during a control period averaged 12.2±2.7 ml/min, 14.5±1.5 ml/min, and 22.9±2.1 ml/min in intact puppies, TPTX puppies and TPTX adult dogs, respectively. The glomerular filtration rate was unaffected by the injection of ECT. Mean values of the weight of kidneys were 17.2±3.1 g in intact puppies, 19.2±1.8 g in TPTX puppies and 26.3±1.7 g in adult TPTX dogs.

Effect of ECT on plasma electrolytes: Concentrations of calcium and phosphorus in plasma samples taken at intervals of 10 to 15 min from dogs equilibrated for 120 min or longer after the operation did not signifi-
cantly differ. Therefore, mean values of the concentrations of three samples obtained from TPTX dogs were taken as controls for the experiments with ECT.

Plasma calcium concentrations were lowered by thyro-parathyroidectomy from 4.8±0.1 to 4.3±0.2 mEq/I in puppies (P<0.05) and from 4.4±0.1 to 3.7±0.2 mEq/I in adult dogs (P<0.05). Plasma calcium concentrations gradually decreased from 4.9±0.4 to 3.8±0.3 mEq/I in intact puppies (P<0.01) and from 4.3±0.2 to 3.3±0.2 mEq/I in TPTX puppies (P<0.01) 120 min after the injection of an ECT dose of 50 MRC U/kg. A significant decrease in the plasma calcium concentration was obtained 15 min after injection of ECT in TPTX puppies and after 30 min in intact puppies (Table 1). On the other hand, injection of ECT caused a slight reduction in the plasma concentration of calcium in 3 out of 4 adult dogs. The mean concentration of calcium fell from 3.7 mEq/I to 3.4 mEq/I in these 4 adult dogs 120 min after the injection of 50 MRC U/kg, the difference being insignificant.

The plasma phosphorus concentration fell from 5.9±0.6 mg/dl to 4.0±0.6 mg/dl, 120 min after ECT (50 MRC U/kg) injection in intact puppies. This change was significant (P<0.01; Table 1). Plasma phosphorus concentrations were raised by thyro-parathyroidectomy in puppies from 5.0±0.4 to 6.8±0.2 (P<0.05), but in adult dogs from 4.1±0.3 to 4.3±0.7. In TPTX puppies and adult dogs, the concentrations of plasma phosphorus were not altered by ECT injection.

Plasma concentrations of sodium and potassium were not altered by intravenous injection of ECT (50 MRC U/kg) in intact puppies. TPTX puppies and TPTX adult dogs. The effects on plasma calcium and phosphorus are summarized in Table 1.

Effect of ECT on urinary electrolytes (Table 2): The injection of ECT (50 MRC U/kg) did not alter the urine flow rate during an observation period of 120 min. The urine flow rate during a control period was 0.29±0.05 ml/min in intact infant beagles, 0.17±0.03 ml/min in TPTX infant beagles and 0.74±0.32 ml/min in adult beagles.

Control values of calcium excretion in intact puppies. TPTX puppies and TPTX adult beagles were 0.88±0.38 μEq/min, 0.33±0.12 μEq/min and 1.1±0.4 μEq/min, respectively. In intact and TPTX puppies, urinary calcium excretion rapidly decreased by 40% and 45%, respectively, 15 min after ECT injection. The decrease of calcium excretion persisted. Similar results were obtained in 3 out of 4 TPTX adult dogs.

Urinary phosphorus excretion increased in intact puppies from a control value of 42.8±11.3 μg/min. Significant increments were obtained 30 min and 60 min after the ECT injection. In TPTX puppies, phosphorus excretion was not significantly altered from the control value of 26.5±8.7 μg/min. In TPTX adult dogs, the phosphorus excretion decreased from a value of 74.8±34.7 μg/min.

Urinary excretion of sodium and potassium was not increased by ECT (50 MRC U/kg) in puppies and adult dogs during an observation period of 120 min. Control values of sodium excretion in intact puppies, TPTX puppies and TPTX adult dogs were 29.2±4.2 μEq/min, 14.0±3.9 μEq/min and 128.4±61.7 μEq/min, respectively; those of potassium excretion were 7.4±2.8 μEq/min, 8.4±1.2 μEq/min and 17.7±2.0 μEq/min, respectively.

DISCUSSION

The present study proved that the extirpation of the thyroid and parathyroid glands decreased plasma calcium concentrations and increased plasma phosphorus concentrations in mature and immature beagles, the increase in the phosphorus concentration being greater in puppies. It is widely known that PTH raises plasma
Table 1. Changes of plasma calcium and phosphorus after EEL calcitonin injection (50 MRC U/kg) in dogs

|                  | 15 min     | 30 min     | 60 min     | 90 min     | 120 min    |
|------------------|------------|------------|------------|------------|------------|
|                  | Intact infant beagle (N=5) |            |            |            |            |
|                  | $P_{Ca}$   |            |            |            |            |
|                  | (mEq/l)    |            |            |            |            |
| $P_{Ca}$         | -0.10±0.06 | -0.26±0.05*| -0.50±0.11*| -0.82±0.09**| -1.06±0.15**|
|                  | (mg/dl)    |            |            |            |            |
| $P_{Pr}$         | -0.06±0.11 | -0.20±0.13 | -0.37±0.12*| -0.79±0.07**| -1.02±0.10**|
| TPTX infant beagle (N=6) |            |            |            |            |            |
|                  | $P_{Ca}$   |            |            |            |            |
|                  | (mEq/l)    |            |            |            |            |
| $P_{Ca}$         | -0.21±0.07*| -0.34±0.10*| -0.59±0.10*| -0.78±0.11**| -0.94±0.10**|
|                  | (mg/dl)    |            |            |            |            |
| $P_{Pr}$         | 0.09±0.18  | 0.00±0.17  | -0.14±0.11 | -0.05±0.19 | -0.14±0.11 |
| TPTX adult beagle (N=4) |            |            |            |            |            |
|                  | $P_{Ca}$   |            |            |            |            |
|                  | (mEq/l)    |            |            |            |            |
| $P_{Ca}$         | -0.02±0.13 | -0.05±0.10 | -0.15±0.15 | -0.31±0.16 | -0.38±0.22 |
|                  | (mg/dl)    |            |            |            |            |
| $P_{Pr}$         | -0.06±0.13 | -0.06±0.12 | -0.07±0.29 | -0.09±0.23 | -0.02±0.26 |

Abbreviations are defined as: TPTX, thyroparathyroidectomized; $P_{Ca}$, plasma calcium level; $P_{Pr}$, plasma phosphorus level. All values indicate the mean±SE. *Significantly different from the zero value; P<0.05. **Significantly different from the zero value; P<0.01.

Table 2. Percent change of urinary electrolytes after EEL calcitonin injection (50 MRC U/kg, i.v.)

|                  | 15 min     | 30 min     | 60 min     | 90 min     | 120 min    |
|------------------|------------|------------|------------|------------|------------|
|                  | Intact infant beagle (N=5) |            |            |            |            |
|                  | $U_{Ca}V$  |            |            |            |            |
| $U_{Ca}V$        | -48.8±6.1**| -48.3±3.7**| -50.5±14.0*| -40.3±12.1*| -48.0±11.7*|
|                  | $U_{Pr}V$  |            |            |            |            |
| $U_{Pr}V$        | 18.8±9.7   | 48.0±6.1** | 38.3±16.3* | 46.9±20.3  | 63.6±26.4  |
|                  | $U_{Na}V$  |            |            |            |            |
| $U_{Na}V$        | -44.8±8.4**| -44.3±9.6**| -36.0±19.8 | -13.0±19.8 | -7.0±22.9  |
|                  | $U_{K}V$   |            |            |            |            |
| $U_{K}V$         | -25.0±12.5 | -15.9±10.2 | -24.0±18.6 | 5.1±16.1   | 23.2±29.2  |
| TPTX infant beagle (N=6) |            |            |            |            |            |
|                  | $U_{Ca}V$  |            |            |            |            |
| $U_{Ca}V$        | -44.7±6.6**| -45.3±8.0**| -49.9±7.0**| -56.9±7.0**| -49.6±7.9**|
|                  | $U_{Pr}V$  |            |            |            |            |
| $U_{Pr}V$        | -16.3±14.0 | -8.2±23.9  | -6.6±36.5  | 30.3±43.6  | 31.8±43.5  |
|                  | $U_{Na}V$  |            |            |            |            |
| $U_{Na}V$        | -32.5±3.0**| -16.8±14.5 | -9.6±13.0  | -22.7±6.8* | -2.8±25.6  |
|                  | $U_{K}V$   |            |            |            |            |
| $U_{K}V$         | 2.0±7.1    | 8.0±10.8   | 18.2±14.6  | 30.0±20.1  | 47.9±20.6  |
| TPTX adult beagle (N=4) |            |            |            |            |            |
|                  | $U_{Ca}V$  |            |            |            |            |
| $U_{Ca}V$        | 6.1±21.7   | 13.3±12.7  | 21.9±19.4  | 14.9±33.3  | 2.9±37.2   |
|                  | $U_{Pr}V$  |            |            |            |            |
| $U_{Pr}V$        | -23.2±7.6  | -31.9±10.6 | -47.6±9.5**| -56.5±8.8**| -55.8±10.1**|
|                  | $U_{Na}V$  |            |            |            |            |
| $U_{Na}V$        | 7.4±18.9   | 1.9±21.2   | 16.0±19.9  | 17.9±25.5  | 6.9±21.0   |
|                  | $U_{K}V$   |            |            |            |            |
| $U_{K}V$         | -6.1±5.6   | 3.4±9.6    | 3.2±7.1    | -0.2±9.7   | -2.3±10.4  |

Abbreviations are defined as: TPTX, thyroparathyroidectomized; $U_{Ca}V$, urinary calcium excretion; $U_{Pr}V$, urinary phosphorus excretion; $U_{Na}V$, urinary sodium excretion; $U_{K}V$, urinary potassium excretion. All values indicate the mean±SE. *Significantly different from the zero percent change; P<0.05. **Significantly different from the zero percent change; P<0.01.
calcium concentrations by increasing calcium resorption from bone (7, 8) and reduces plasma phosphorus concentrations by decreasing renal tubular reabsorption (9). In mature and immature beagles, PTH appears to function predominantly in maintaining plasma calcium concentrations, as compared with the calcium-decreasing action of endogenous calcitonin.

The hypocalcemic effect of ECT was greater in puppies than in adult beagles. Maximum reduction of plasma calcium caused by ECT did not differ in intact and TPTX puppies; however, the counter effect of endogenous PTH appears to be reflected in the slower development of hypocalcemia in intact puppies (Table 1). According to Tashjian (10), the hypocalcemia induced by calcitonin is prolonged in rats following thyroid-parathyroidectomy. The hypocalcemia induced by intravenous injections of calcitonin is reportedly reduced with advancing age in rats (11-13). Orimo and Hirsch (14) have demonstrated that the hypocalcemic effect of endogenous calcitonin released by thyroid cautery, and the exogenous calcitonin is also decreased with increasing age in rats. The present study clearly demonstrated a similar dependence of calcitonin actions on age in dogs. In the present study, no functional difference in GFR and RBF per kidney weight was observed in infant and adult beagles. Further, there is no difference in the half life of plasma ECT between immature (4 week old) and mature (15 week old) rats (15). Therefore, the possibility for a different rate of ECT clearance from plasma in mature and immature dogs and rats may be excluded. The more likely explanation is that the rate of bone metabolism is reduced with age.

ECT injections rapidly decreased the urinary excretion of calcium before the plasma calcium level was lowered, suggesting that the hypocalcemia is not associated with an increased calcium excretion, and the attenuation of calcium excretion is not due to decreased plasma calcium. Thus, ECT in the dose used here appears to act directly on kidneys and reduce renal excretion of calcium. In contrast, calcitonin produces hypercalcemia in humans (15-18) and sheep (19), possibly by its action on renal tubular reabsorption. The fact that the plasma calcium was lowered despite a decrease in calcium excretion suggests that ECT interferes with bone resorption of calcium and may promote calcium bone deposition (20).

In intact puppies, plasma phosphorus decreased and urinary phosphorus excretion tended to increase by ECT, whereas in all TPTX dogs, phosphorus excretion was unaffected or decreased. These data are in agreement with the result using porcine calcitonin in adult dogs as reported by Pak et al. (21). In contrast, ECT did not increase the excretion of sodium in intact and TPTX puppies. In dogs, PTH reduces sodium and phosphate reabsorption along the proximal tubule (22); however, sodium but not phosphate is reabsorbed at distal nephrons. These findings may indicate that PTH is not significantly involved in the sodium excretion, but hypophosphoremia and phosphaturia induced by ECT only in intact beagle puppies appears to be due to an increases in PTH secretion, being possibly associated with hypocalcemia. Calcitonin does not act directly on dog kidney to cause natriuresis (present study, 23). On the other hand, in rats, salmon (24), and porcine (25, 26), calcitonin increases sodium excretion. In these cases, calcitonin may possibly have an action on the kidneys since intact and TPTX rats responded to calcitonin with a significant increase in sodium excretion.

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