Changes Due to Age in the Hypocalcemic and Hypophosphatemic Effects of Salmon Calcitonin in Growing Rats[1] (37517)

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There have been numerous reports demonstrating a decreasing hypocalcemic effect of thyrocalcitonin with age in several species (1–4). In addition Milhaud, Perault-Staub and Moukhter (5) reported that the ability of thyrocalcitonin to lower both calcium and phosphate concentrations was less pronounced in rats over 110 days of age than in younger rats.

The hypophosphatemic effect of both porcine thyrocalcitonin (PCT) and salmon calcitonin (SCT) has been the subject of considerable study in our laboratories. We have been able to demonstrate that the hypophosphatemic effect of the calcitonins was primarily due to their ability to move phosphate out of plasma (6, 8), in contrast to the hypocalcemic effect of the hormone produced by inhibition of the entry of calcium into plasma (9, 10).

Because of these contrasting actions of the calcitonins on plasma calcium and phosphate concentrations, it seemed pertinent to compare in more detail the effect of age on the hypophosphatemic and hypocalcemic action of SCT.

Materials and Methods. Approximately 150 male Zivic-Miller rats, maintained until use on Purina Laboratory Chow were utilized for these experiments. Some of the older rats were reused in tests several weeks apart. The age of the rats ranged from 37 to 138 days. For each individual experiment, both control rats and those receiving calcitonin were born on the same day. The same lot of SCT (kindly supplied and assayed by Dr. J. W. Bastian of Armour Pharmaceutical Laboratories) was used throughout the experiment. The SCT (590 MRC U/mg) was diluted with acetate buffer and albumin and injected subcutaneously into each rat as a single dose of 0.6 MRC mU/g body weight, at a pH of 3.0.

Other than the age of the rats, the protocol for all experiments was identical. The evening before SCT administration the animals were fasted and supplied with distilled H_2O for drinking. Between 8 and 9 AM the following morning, ^{32}P was injected intravenously (via jugular) under ether anesthesia. One hour later, a blood sample was obtained (from the tail) and SCT was injected. Blood samples (0.35 ml) were obtained at hourly intervals thereafter for 4 hr. In the last two experiments, the time course was extended to 7 hr, and in one, SCT effects were compared with those of the PCT preparation also provided by Armour Laboratories (Lot K297185, 160 MRC U/vial).

Two methods for calcium and phosphate analysis were used interchangeably except that for any single experiment all analyses for each ion were done by the same method. One method utilized the Beckman discrete sample analyzer measuring calcium and phosphate by modified procedures (11, 12) in 10 μl samples of plasma. For the other method, calcium was analyzed fluorometrically by the method of Hill (13), and phosphate colorimetrically by the method of Chen, Toribara and Warner (12). All ^{32}P measurements were made in a 10% TCA-filtrate of plasma using liquid scintillation procedures previously described (8).

Statistical analyses were done by Student's 2-tailed t test.

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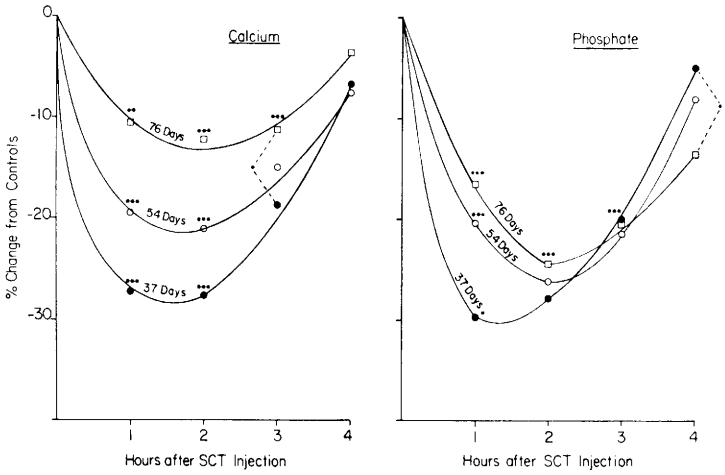

FIG. 1. Changes in plasma calcium and phosphate concentrations following SCT injection in 37–76 day old rats. Change (%) from controls plotted against hours after SCT injection. Each point represents the comparison between 8 controls and 8 SCT-injected animals. Statistical comparisons between adjacent vertical points on each graph are given by asterisks. The asterisks above the top line are for comparison to noninjected controls (zero on the graph). *** $p < .0005$; ** $p < .025$; * $p < .05$.

Results. SCT-produced plasma calcium and phosphate change, ages 37-76 days. For the major group of experiments, comparison was made between the hypocalcemic and hypophosphatemic effects of SCT in rats of three age groups, 37, 54, and 76 days. The plasma calcium and phosphate changes are summarized in Fig. 1 as percentage change from controls run concurrently. The marked decrease with age in the resulting hypocalcemia confirms previous studies (1, 5). As the rats grew older, the depth of hypocalcemia produced by SCT was reduced, and plasma calcium values tended to return toward normal sooner as has been reported by Copp and Kuczerpa (3). The change in the hypophosphatemic effect with age is less obvious. At 1 hr after SCT injection, there was a statistically significant decrease in the hypophosphatemia as a function of increasing age of the rat, though to a lesser degree than for the hypocalcemic effect. Examination of the 4 hr period indicated, however, that this change in hypophosphatemia was primarily a postponement of both the time of the maximum fall in plasma phosphate concentrations and in the time of recovery. Superimposed on this delay was a slight reduction in the maximum decrease in plasma phosphate in the older rats. Even though statistical analyses of the area above each curve to the zero level in Fig. 1 was not made, it is obvious that the subtended area decreases markedly with each successive age for the hypocalcemic effect. The decrease for the corresponding hypophosphatemic curves is much less, indicating that the change with age for the two effects is different.

In order to compare these changes in another way the data were replotted as the percentage change from controls for the SCT-injected rats as a function of the age of the rat for each hour after SCT administration (Fig. 2). This method of presentation permits a comparison of the changes in the hypocalcemic and hypophosphatemic effects. It is readily apparent that the ratio, $\Delta Ca:\Delta PO_4$, decreases markedly with age under these standardized conditions and during this age span.

Effect of age on the influence of calcitonin on plasma ^{32}P disappearance curves. For each age group, the effect of SCT on the rate of ^{32}P disappearance (injected 1 hr prior to SCT) from plasma was determined. Since these data, when graphed as percentage change from controls run concurrently, were

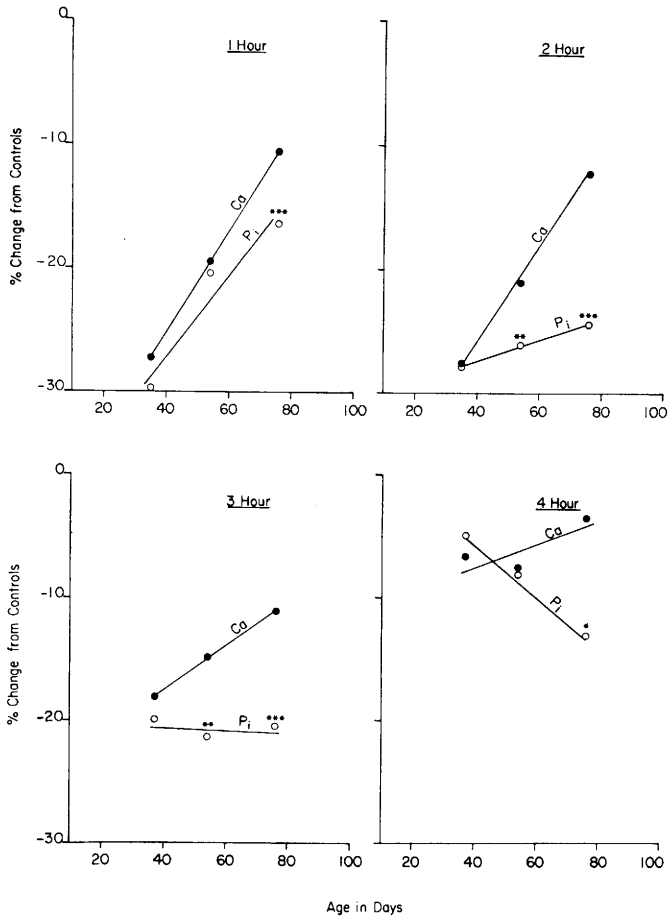

FIG. 2. Changes in plasma calcium and phosphate concentrations following SCT injection in 37–76 day old rats. Change (%) from controls plotted against age of rats. Key for each point and statistical comparisons is the same as for Fig. 1.

not statistically different from the phosphate data shown in Fig. 1, they are not produced here. The data confirm the previous report (7) that while SCT increased the rate of ^{32}P disappearance from plasma, the relative changes between SCT-injected and controls mirrored those for stable phosphate. Therefore SCT did not, under these conditions, affect plasma ^{32}P specific activity curves.

SCT-produced hypophosphatemia and hypocalcemia in rats aged 110–124 days. The effect of SCT in this age group is summarized in Fig. 3. The hypophosphatemia produced followed the pattern of that of the younger rats. The plasma phosphate curve in Fig. 3 could readily be plotted with the other age groups in Fig. 1. The difference in this age group, however, is seen in the calcium data. The failure of the plasma calcium concentrations to return to normal during the experimental period was in contrast to the effects in the younger rats. Since this was an unexpected finding, the experiment was repeated several times and each point on the curve represents the mean of 24 experimental and 24 control rats.

Comparison of SCT and PCT in 138 day old rats. It was considered that the prolongation of the hypocalcemic effects seen in the 110–124 day old rats might be a characteristic of the type of calcitonin used (SCT). Habener *et al.* (14) have shown that SCT is cleared 10 times more slowly from circulation than the mammalian hormone. A comparison

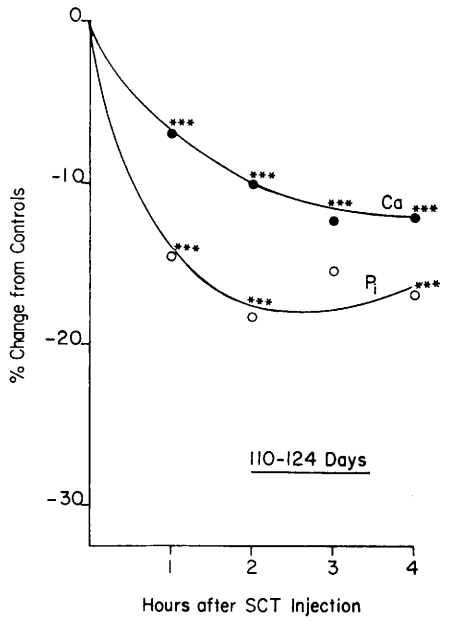

FIG. 3. Changes in plasma calcium and phosphate concentrations following SCT injection in 110–124 day old rats. Each point represents the comparison between 24 controls and 24 SCT-injected animals. For statistical analysis see Fig. 1.

was, therefore, made between the effect of SCT (0.6 MRC mU/g) and PCT (0.4 MRC mU/g) on these parameters in one group of rats used previously but now 138 days of age. The data are summarized in Fig. 4. While approximately the same dose level of the two types of calcitonins were administered, they were not re-assayed in our laboratory, and therefore it cannot be assumed that the same potency of each type was administered. However, there appears little doubt that the prolonged effects demonstrated in the experiments summarized above are more a characteristic of salmon calcitonin than of porcine thyrocalcitonin. This experiment also demonstrates that the difference between the hypocalcemic and hypophosphatemic effects relative to age is not solely a characteristic of salmon calcitonin, since in this age group only the hypophosphatemic effect was evident following PCT injection.

Discussion. These studies reconfirm the observation that during the aging process in the growing adolescent and young adult rat, there was a decrease in the hypocalcemia produced by calcitonin. This change with age (up to 100 days) was represented by a decrease in the depth of hypocalcemia and in its duration. A different type of change relative to age was seen in the hypophosphatemia produced by SCT. In this parameter the major changes were a delay with age in the time necessary to produce the maximum hypophosphatemia, and a corresponding delay in the recovery from the single injection of the hormone. Accompanying these changes was a small decrement in the depth of the hypophosphatemia produced.

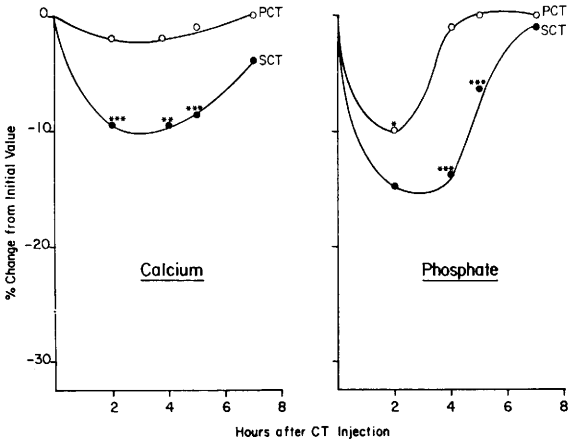

FIG. 4. Changes in plasma calcium and phosphate concentrations following SCT and PCT injection in 138 day old animals. Each point represents the comparison between 8 SCT-treated and 7 PCT-treated animals. For statistical analysis see Fig. 1.

It must be emphasized that these studies were confined to the adolescent and young adult male rat. No evidence is provided concerning the hypophosphatemic effect of SCT in the aged rat. The important point to be made from this study is that the changes in the response to SCT relative to the ages examined are different for phosphate than for calcium. These data add further evidence for the hypothesis, therefore, that the effect of calcitonin on plasma calcium and plasma phosphate concentrations is the reflection of distinct and separate physiological responses. A second point is that in each age group studied (37–138 days), the effects of SCT on plasma ^{32}P mirrored those on stable phosphate. These results support the conclusion that an immediate effect of this hormone in the rat is to move phosphate out of plasma.

Finally, these studies provide an additional demonstration that salmon ultimobranchial calcitonin has a longer duration of action in mammals than do purified preparations obtained from mammalian thyroid glands. These data also suggest that the hypocalcemic response to SCT in rats changes between the age of 75 and 110 days of age. No explanation of this change in response can be given.

Summary. These studies have compared the hypocalcemic and hypophosphatemic effects of salmon calcitonin (SCT) in male rats between the ages of 37 and 138 days. In addition, the ability of SCT to increase the rate of disappearance of ^{32}P (injected 1 hr prior to SCT) was also compared for these ages. SCT was administered subcutaneously at a constant dosage of 0.6 MRC mU/g body weight. Previously reported changes in the hypocalcemic effect of porcine thyrocalcitonin (PCT) with age were confirmed by these studies with SCT; namely a decrease in both the depth of the hypocalcemia produced and its duration. In contrast, changes with age in the hypophosphatemic effect of SCT were: (a) a delay in the time after hormone injection at which the maximum hypophosphatemia was produced; and (b) a delay in the time of recovery from the single injection. At all ages, the effect of SCT on plasma ^{32}P disappearance rates mirrored those on stable phosphate. It is suggested that these studies add further evidence that the hypocalcemia and hypophosphatemia produced by SCT are the reflection of different physiological responses; and that the effect of SCT on phosphate is to produce a rapid movement of this ion from plasma, in contrast to its inhibition of calcium entry into plasma.

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