Pyrophosphate Enhancement of Hypocalcemic Effect of Thyrocalcitonin in Rat

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Synopsis

Enhancing effect of neutral phosphate on the hypocalcemic response to thyrocalcitonin (TC) has been previously demonstrated in rats. The possibility of in vivo pyrophosphate enhancement of TC effect was investigated in the present study. Marked dose-dependent hypocalcemia was found 60 min. after s.c. injection of graded doses of pyrophosphate (4 – 1,024 μ mols) in both intact and thyroparathyroidectomized rats. At all dose levels of TC used simultaneously injected pyrophosphate markedly enhanced the 60 min. hypocalcemic response to graded doses of TC.

Thyrocalcitonin (TC) exerts its hypocalcemic effect through an action on bone (Munson et al., 1966). During the last three years, a number of studies in vivo have indicated that this effect is due to inhibition of bone resorption (Johnston et al., 1966; Milhaud et al., 1968; Wallach et al., 1967; Martin et al., 1966; O’Riordan et al., 1966). The administration of phosphate has also been shown to lower blood calcium probably through increasing deposition of bone salt (Pechet, M. M. et al., 1967). According to the recent report of Hirsch (1968), the administration of phosphate markedly enhanced the hypocalcemic response to TC in rats. In the present report, we have studied the enhancement of TC effect by pyrophosphate in rat.

Materials and Methods

Male Wistar rats of 100 – 150 g of the same age were maintained on Oriental Rat chow unless otherwise specified and then fasted overnight before use. Solutions of sodium pyrophosphate ranging in concentration from 4 – 1,024 μ mols were injected s.c. alone or with TC at a separate site. Blood samples were obtained by cardiac puncture at the intervals indicated in each experiment and the serum calcium was determined by the colorimetric autoanalyzer method of Gitelman (1967). TC with the potency of 20 MRC units/mg was dissolved in 0.1 M Na acetate containing 0.1% albumin before use. The data in each experiment were subjected to analysis of variance. The standard errors were obtained from the residual error term of the analysis of variance. The means were compared using the multiple comparison test of Hartley (1967).

Results

Hypocalcemic response to graded doses of pyrophosphate

Hypocalcemic response was obtained 1 hr. after s.c. injection of pyrophosphate (4 – 1,024 μ mols) in young rats fasted for 1 day as shown in Figure 1. Dose dependent hypocalcemia along with increasing dose of pyrophosphate is evident. Such hypocalcemia was almost the same with phosphate induced hypocalcemia when corresponding doses in terms of phosphorus content were used.
Duration of hypocalcemic response to pyrophosphate in young rat

Young fasted rats were injected s.c. with 120 μ mols of pyrophosphate and blood was drawn from separate groups of animals before and at 0.25, 0.5, 1.0, 3.0 and 5.0 hr. after the injection. As shown in Figure 2, a significant hypocalcemia developed 0.5 or 1.0 hr. after the injection of pyrophosphate.

Hypocalcemic effect of graded doses of pyrophosphate in thyroparathyroidectomized rat maintained on high calcium diet for 2 days

Rats were surgically thyroparathyroidectomized and fed with high calcium diet (Ca content 3.0%) for two days. On the third day, the 60 min. hypocalcemic response to graded doses of pyrophosphate was examined. Figure 3 shows the dose dependent hypocalcemia with increasing dose of pyrophosphate.

Dose response to TC with and without pyrophosphate in young fasted rats

In Figure 4 is shown the 60 min. hypocalcemic response to increasing doses of TC with and without simultaneous injection of 64 μ mols of pyrophosphate in young rats fasted overnight. Although no change occurred in serum calcium upon administration of pyrophosphate alone, pyrophosphate markedly enhanced the hypocalcemic effect of TC at all doses of TC examined.

Duration of response to TC in young rats with and without pyrophosphate

Young fasted rats were injected s.c. with either 64 μ mols of pyrophosphate, 45 mU of TC or both and blood was drawn from separate groups of animals before and at 1.0, 2.0 and 5.0 hr. after the injection. These results are shown in Figure 5.

While pyrophosphate alone caused only small and insignificant changes in serum calcium, a significant hypocalcemia most profound at 2 hr. was observed when TC was administered. Furthermore, the hypocalcemic effect of TC was significantly enhanced by the simultaneous administration of pyrophosphate.

Discussion

It has been shown that the administration of phosphate lowers serum calcium probably through the increase in bone mineralization (Pechet et al., 1967). However, the exact mechanism of such an action is not fully understood. Pyrophosphate also shares such hypocalcemic property according to the results of the present study. Fleisch et al., (1962) are the opinion that pyrophosphate, a normal constituent of plasma, might be important in inhibiting calcification in vivo. We have recently found that the addition of pyrophosphate in the bone culture system markedly inhibited the release of previously incorporated Ca45 into the medium. Since the histological findings also demonstrated the inhibition of bone resorption, these findings strongly suggest the pyrophosphate inhibition of bone resorption (Orimo et al., 1969). In view of these findings, it is tempting to speculate that pyrophosphate decreases serum calcium through the inhibition of bone resorption. TC exerts its hypo-
Fig. 2. Duration and magnitude of the hypocalcemia caused by 120 μ mols of pyrophosphate in young rats fasted overnight. Each point represents the mean of 5 rats.

Fig. 3. Dose response relationship in young thyro-parathyroidectomized rats fed high calcium diet (Ca 3.0%) for two days and injected with graded doses of pyrophosphate. Each point represents the mean of 5 rats.

Fig. 4. Greater 60 min. hypocalcemic response to TC in rats fasted overnight and injected with TC and 64 μ mols of pyrophosphate, compared with that in rats injected with TC alone. Each point represents the mean of 5 rats. Serum calcium values were significantly different between the 2 groups at 45 – 405 MRC mU of TC (p < 0.001).
Fig. 5. Duration and magnitude of the hypocalcemia caused by 45 mU of TC with or without 64 μ mols of pyrophosphate in young rats fasted overnight. Serum calcium value was significantly smaller (p < 0.05) in rats injected TC and pyrophosphate than in rats injected TC alone at 1.0 hr. and 2.0 hr. after the injection.

calcemic effect by inhibiting bone resorption (Munson, P. L. 1966). In the course of experiments designed to develop a sensitive bioassay method for TC, it was found that rats fed a low calcium diet with adequate phosphate were more sensitive to TC than rats fed a diet low in both calcium and phosphate (Cooper et al., 1967). Subsequently, Hirsch (1968) reported that s.c. or i.p. administration of inorganic phosphate markedly enhanced the hypocalcemic response to TC in young rat. More recently phosphate was also found to enhance the effect of TC in young rats fed a high calcium diet and in 1 year old rat (Orimo et al., 1969). Since the bone resorption is supposed to be completely stopped by a high dose of TC alone, the additional hypocalcemia produced by the combination of phosphate and TC must be due to some mechanism other than inhibition of bone resorption. An increase in bone accretion is most likely playing a role as shown by Milhaud (1968) with the use of Ca⁴⁵.

Although the exact mechanism whereby pyrophosphate enhances the hypocalcemic response to TC has not yet been elucidated, there is a possibility that effect of TC is enhanced by the further inhibition of bone resorption. These findings, together with the previous findings with phosphate, should encourage further studies of combination of TC and pyrophosphate in the treatment of diseases characterized by excessive bone loss.

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