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INCREASE IN PLASMA CALCITONIN FOLLOWING FEMORAL FRACTURE IN RATS

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The secretion of calcitonin (CT) has been studied in male rats following a standardized, left femoral fracture after administration of subcutaneous fluanisone and fentanyl citrate anaesthesia. Twenty-five minutes after the fracture, the plasma concentrations of CT were increased by about 20 per cent in young and by about 60 per cent in adult rats compared to prefracture levels of the hormone. Three weeks later, plasma CT had decreased and was not significantly different from prefracture levels.

Anaesthesia combined with femoral fracture did not influence plasma calcium significantly, whereas the plasma concentrations of calcium increased in young control rats during 35 minutes of anaesthesia alone. Plasma CT, however, remained unchanged in these control rats in the same period.

In rats with a transplanted, CT-secreting, medullary thyroid carcinoma, femoral fracture did not alter the already high plasma concentrations of CT.

It is suggested that increase in CT secretion is part of a general response to trauma.

Key words: anaesthesia; calcium; medullary thyroid carcinoma; plasma concentration; wounds and injuries

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Calcitonin (CT) is a hypocalcaemic and hypophosphataemic hormone produced by the parafollicular cells of the thyroid gland (Foster et al. 1964). A major physiological effect of CT is to inhibit bone resorption, while it is unclear whether the hormone also enhances bone formation (Rasmussen & Bordier 1974, Talmage & Cooper 1979). Plasma ionic calcium and one or more intestinal hormones (e.g. gastrin) are postulated as physiological secretagogues for CT (Talmage & Cooper 1979).

We have previously reported that the concentration of CT increased four-fold in plasma of rats during operative manipulation of the kidneys (Ekeland & Gautvik 1979). Since bone is a main target organ for CT action (Simmons 1976), the purpose of the present study was to investigate how bone trauma might influence plasma CT. As young male rats are known to be most sensitive to the hypocalcaemic effect of CT (Rasmussen & Bordier 1974, Simmons 1976), the study was carried out using both young and adult male rats. Also rats with a transplanted, CT secreting tumour and consequently high circulating levels of the hormone were used.

MATERIALS AND METHODS

Young rats

Twenty outbred male Wistar/Af/Han/Mol SPF rats, 23–25 days of age and weighing about 50 grams, were divided into two weight-matched groups of 10 animals. In one group the left femur was fractured as described below. The other group served as control.

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Adult rats

Twenty-five inbred, Wistar-derived, male Wag/Rij rats, 6–7 weeks of age were divided into two weight-matched groups. One group (13 rats) received no further treatment until the present fracture experiment. In the other group (12 rats), a specimen from a medullary thyroid carcinoma (MCT) was transplanted beneath the left kidney capsule under ether anaesthesia, as described by Boorman et al. (1974). The original tumour, which was found in the thyroid of rats kept for ageing studies (Boorman et al. 1972), had previously been serially transplanted beneath the kidney capsule of recipient rats. The MCT specimen used for transplantation in the present experiment represented the fourth generation. In accordance with previous reports (Normann et al. 1977, Ekeland et al. 1980), the transplanted tumour tissue grew and produced CT, raising the circulating levels of the hormone. By the time of the present experiment, the rats were 6 months old, weighing about 320 grams.

Five rats were kept in each cage and they were given standard animal pellets containing 0.9 per cent calcium and 0.7 per cent phosphorus (Bl. nr. 3155, Møllesentralen i/s, Oslo, Norway) and water *ad libitum*. Following subcutaneous anaesthesia (fluanisone 5 mg/kg and fentanyl citrate corresponding to fentanyl 0.1 mg/kg, Hypnorm Veterinary®, Mekos, Helsingborg, Sweden). a standardized, transversal, mid-diaphyseal, left femoral fracture was made in all the rats (except the young controls) with a specially designed forceps (Ekeland et al. 1980). The fractures were left to heal without immobilization. Blood samples (in heparinized tubes) were obtained by cutting the distal tail of the rats 10 minutes before and 25 minutes, as well as 3 weeks, after the fracture. Blood was sampled simultaneously from the young control rats without fractures. All blood samples were obtained during subcutaneous anaesthesia, as described above. The samples were immediately cooled on ice, centrifuged, and the plasma stored at −20°C until analysed for CT and calcium.

Plasma levels of CT were monitored radio-immunologically using rabbit antisera to synthetic human CT–M and ^{125}I labelled CT–M (Gautvik et al. 1976). These antisera are directed against both N-terminal and C-terminal amino acid sequences of the hormone (Myhre & Gautvik 1979). Rat and human CT differ by only two amino acid residues (Raulais et al. 1976), and there are immunological similarities between CT from the two species (Burford et al. 1975). Therefore, this radioimmunoassay using antisera raised against human CT has proved to be a reliable method also for measuring rat CT (Normann et al. 1977, Ekeland et al. 1980).

Plasma calcium was measured by atomic absorption spectrophotometry (Perkin Elmer, model 360, Norwalk, Connecticut, USA), as described by Trudeau & Freier (1967).

Median with 0.25- and 0.75-fractiles were used to express the average and the dispersion of the measured values. The statistical significance probability was calculated by Wilcoxon's two-tailed tests for two samples and pair differences (Diem & Lentner 1975). In rats with transplanted MCT, however, Wilcoxon's one-tailed test for pair differences was used to evaluate increase in plasma CT with time. Differences were considered significant when $2P \leqq 0.05$ (two-tailed test) and $P \leqq 0.05$ (one-tailed test).

RESULTS

Young rats

Twenty-five minutes following femoral fracture, the plasma levels of CT were significantly increased compared both to prefracture levels ($2P < 0.02$) and to CT levels in control rats without fracture ($2P < 0.01$) (Figure 1). Three weeks later, the plasma concentrations of CT in rats with femoral fracture had decreased, and were not significantly different from prefracture levels or from hormone concentrations in control rats.

No significant alterations were observed in plasma calcium following femoral fracture in

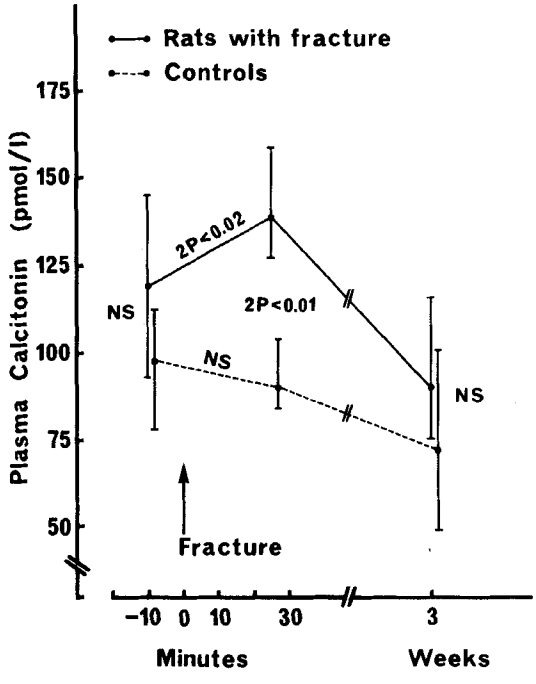

Figure 1. Plasma levels of calcitonin in young rats 10 minutes before and 25 minutes, as well as 3 weeks, after femoral fracture. Corresponding levels in control rats are also shown. Ten animals in each group. NS = Not significant. (Median with 0.25- and 0.75-fractiles).

Table. 1. Plasma calcium (mmol/l) before and after left femoral fracture in young and adult rats. The levels of significance for horizontal and vertical comparisons are indicated. N.S. = Not significant. n = Number of animals. (Median with 0.25- and 0.75-fractiles)

Animals	n	Time before/after fracture				
		10 minutes before		25 minutes after		3 weeks after
Young rats with fracture	10	2.23 (2.05–2.30)	NS	2.37 (2.23–2.47)	NS	2.42 (2.12–2.45)
		NS		NS		NS
without fracture (controls)	10	2.18 (2.15–2.28)	$2P=0.01$	2.62 (2.30–2.65)	NS	2.63 (2.36–2.65)
Adult rats with fracture	4–5	2.42 (2.37–2.47)	NS	2.41 (2.33–2.54)	NS	2.55 (2.47–2.82)

young rats, either when compared to prefracture levels, or to corresponding levels in control rats (Table 1). The plasma calcium concentrations in control rats, however, increased significantly ($2P = 0.01$) from the first to the second sampling of blood.

The body weights of rats with fractures and the controls were not significantly different, and the weights were about 210 grams at the end of the experiment.

Adult rats

Plasma CT was increased by about 60 per cent compared to prefracture levels of the hormone ($2P < 0.01$) 25 minutes after femoral fracture in normal Wag/Rij rats (Figure 2). Three weeks later, prefracture levels of plasma CT were again observed. In contrast, femoral fracture did not change the already high circulating levels of CT in Wag/Rij rats with a transplanted MCT (Table 2). Growth of the tumours progressed as time went by, and 3 weeks after the fracture, the plasma levels of the hormone were almost doubled.

Plasma calcium was not influenced by femoral fracture in normal Wag/Rij rats (Table 1). In corresponding rats with a CT producing tumour, the plasma samples obtained were too small to measure calcium. Earlier experiments in these rats have, however, shown that total plasma calcium is unaffected by the growth of the tumour (Ekeland et. al. 1980).

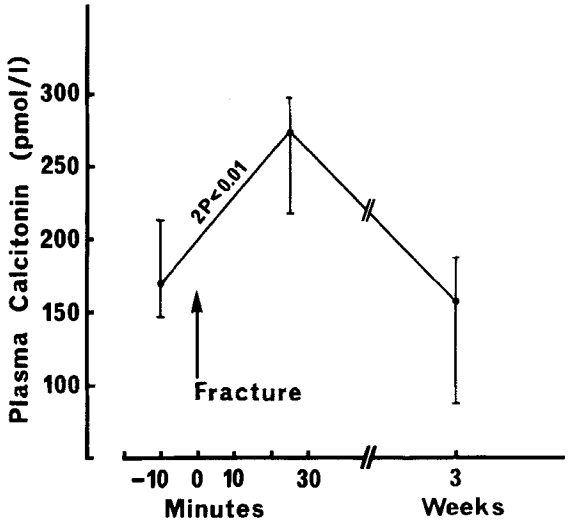

Figure 2. Plasma levels of calcitonin in adult rats 10 minutes before and 25 minutes, as well as 3 weeks, after femoral fracture. Thirteen animals in the two first groups, 6 in the latter group. (Median with 0.25- and 0.75-fractiles).

There were no significant differences in body weights between Wag/Rij rats with and without transplanted MCT, the weights being about 310 grams 3 weeks after the fracture.

DISCUSSION

Already 25 minutes after femoral fracture, the plasma concentrations of CT were increased by

Table 2. Plasma calcitonin (CT) (pmol/l) before and after left femoral fracture in rats with a transplanted, CT secreting tumour. Levels of significance for comparisons are indicated. NS = Not significant. n = Number of animals. (Median with 0.25- and 0.75-fractiles)

	Time before/after fracture				
	10 minutes before $n=12$		25 minutes after $n=12$		3 weeks after $n=6$
Plasma CT	660 (475–808)	NS	631 (452–709)	$P<0.025$	1129 (999–1792)

about 20 per cent in young and by about 60 per cent in adult rats compared to prefracture hormone levels. This rapid increase in circulating CT following a trauma is in agreement with an earlier study (Ekeland & Gautvik 1979), and suggests a stimulation of CT secretion. The different magnitude of changes in plasma CT in the two age groups in the present study may be related to the normally low CT secretion in young as compared to adult male rats (Deftos 1979). However, differences between the outbred Wistar rats and the inbred Wistar derived Wag/Rij rats in their response to CT secretion stimuli might also be considered.

CT is a hypocalcaemic hormone, but the observed increase in plasma CT following femoral fracture did not change the plasma concentrations of calcium significantly. Plasma calcium is regulated by the action of several hormones whose secretion may also change following anaesthesia and trauma. Thus, the secretion of parathyroid hormone (PTH) has been reported to increase following operative bone trauma in humans (Hulth & Johnell 1979). The parathyroid response to secretion stimuli is rapid (Chambers et al. 1979), and it may thus be possible that the secretion of both CT and PTH increase within half an hour following femoral fracture, and that their opposite effects on circulating calcium leave the plasma concentration of this mineral unchanged. It has previously been reported that catecholamines stimulate CT and PTH secretion both in rats (Hsu & Cooper 1975) and humans (Vora et al. 1978, Kukreja et al. 1975), possibly through activation of adrenergic β_2 receptors (Fournier et al. 1979). As secretion of catecholamines increases following trauma (Kiyomi & Brooks 1974), this may have influenced both the CT and PTH secretion in the present study.

Plasma calcium concentrations increased in young control rats during 35 minutes of fluanisone and fentanyl citrate anaesthesia (Table 1), while the CT levels in plasma were unchanged (Figure 1). In corresponding rats subjected to the same anaesthesia and femoral fracture, the levels of CT in plasma increased while plasma calcium remained unchanged. This may indicate that calcium regulating factors other than CT are activated during anaesthesia in rats, leading to increase in plasma calcium. Thus, in rats submitted to both anaesthesia and fracture, this rise in plasma calcium would be prevented by the concomitantly increased CT secretion.

In a previous study in rats with a transplanted MCT, the tumour cells had retained the ability to increase CT secretion in response to pentagastrin and calcium injections (Normann et al. 1977). The circulating immunoreactive CT was also biochemically similar to intact CT and biologically active (Myhre et al. 1979, Ekeland et al. 1980). Rats with a transplanted MCT should therefore be a suitable animal model to study CT secretion following stimulation. However, the plasma concentrations of CT did not change following femoral fracture in these tumour bearing rats, in contrast to plasma CT in corresponding rats without tumour. Prefracture plasma CT levels in Wag/Rij rats with tumour (Table 2) were more than twice the postfracture levels of the hormone in Wag/Rij rats without tumour (Figure 2). Thus, femoral fracture in rats with MCT was perhaps

not a sufficient stimulus to provoke an increase of plasma CT over and above the already high circulating levels of the hormone.

The observed increase in plasma calcitonin after femoral fracture is probably secondary to the trauma, rather than to specific effects due to functional impairment of a target organ (bone). Thus, increased circulating CT has also been observed during operative manipulation of rat kidneys (Ekeland & Gautvik 1979), and after operative and other severe traumas in humans (Jørgensen et al. 1979, Lennquist et al. 1979a, b).

In conclusion, it seems likely that the plasma CT increase following femoral fracture in rats is part of a general response to trauma. The possible functional implication of this observation remains, however, unclear.

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