Influence of α-MSH and ACTH on cortical bone remodelling in hypophysectomized rats

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Summary. Hypophysectomy increases both periosteal resorption and endosteal apposition along the femur diaphysis in rat. Administration of α-MSH decreased the periosteal resorption but had no effect on the endosteal apposition. ACTH had only minor effects on the endosteum. Thus, α-MSH and ACTH, in the doses used, have different effects on cortical bone in rat. The effect of α-MSH on cortical bone could be an effect of the hormone alone or by its stimulation of other factors.

The effect of α-MSH on cortical bone remodelling has not previously been investigated and little is known about the influence of α-MSH on mineralized tissues. Administration of MSH to rabbits causes a decrease in serum-Ca which might be due to an increased inflow of calcium to the skeleton[1,2]. In hypophysectomized rats the administration of α-MSH does not stimulate the longitudinal growth in proximal tibia, contrary to growth hormone and thyroxine[3,4]. According to Bernasconi et al.[5] and Runnebaum et al.[6], α-MSH has adrenocorticotrophic properties but this has not been confirmed in other investigations[7-9].

This investigation studied the effect of α-MSH and ACTH on cortical bone remodelling along the femur diaphysis in hypophysectomized female rats. Tetracycline was used as an intravital marker to study the remodelling process[10].

Materials and methods. Female rats (Sprague-Dawley) were delivered from the breeding-farm 1 week before the investigation period for acclimatization. All had a known date of birth, registered as day 1. They were kept under standardized conditions and fed on pellets and water ad libitum. On arrival and during the experiments, their general condition and weight were registered.

The following groups were used:

group I 12 rats - normal controls;
group II 20 hypophysectomized rats. No hormone administration - hypophysectomized controls;
group III 12 hypophysectomized rats given 1 or 20 µg/day and animal of α-MSH for 20 days.
group IV 11 hypophysectomized rats given 10 or 20 µg/day and animal of α-MSH for 10 days.
group V 6 hypophysectomized rats which received 2 IU/kg ACTH/day and animal for 10 days.

Hypophysectomy was made at age 60 days by the parapharyngeal approach[11-13] under ether anaesthesia. Cortisone acetate, 0.5 mg/kg, was igen s.c. at the time of hypophysectomy to increase the survival rate[13]. The normal animals received no cortisone. The completeness of the hypophysectomy was determined as described previously[13].

After a 15-day postoperative control period, α-MSH or ACTH were administered s.c. either during age 75–85 days, followed by a 10-day withdrawal period, or during age 75–95 days. The animals were killed at age 95 days.

To determine the rate of appositional bone formation and resorption in the femur diaphysis, single doses (10 mg/kg) of oxytetracycline, OTC, were administered i.p. under ether anaesthesia at age 60 and 75 days. The animals were killed at age 95 days.

The right femur was used to determine the periosteal and endosteal growth and resorption, as described earlier[10]. The femur diaphysis was cut by a grinding plate into transversal sections of about 100 µm in thickness. Measurements were made along the femur diaphysis in the ventrolateral part of the cortical circumference[10]. in incident light in a fluorescence microscope.

Measuring technique. Instead of using calipers to determine the different section levels along the femur diaphysis, the distance between the fluorescent bands deposited endosteally at age 60 and 75 days was used as reference distance (E_{60}-E_{75}) to get comparable values in hormone-treated and control animals (hypophysectomized and normal) as this distance is the same[14]. This distance was earlier shown[10] to increase consistently the further distal along the femur diaphysis the sections are taken. Thereby sections with the same reference distance, i.e. the same endosteal growth during age 60–75 days, are compared. This is possible as the experimental conditions in hypophysectomized control animals and in hormone-treated hypophysectomized animals are the same during age 60–75 days. Thus, the corresponding osteoblast and osteoclast level along the diaphysis is compared.

Measurements were also made from the endosteal OTC-band deposited at age 60 days (E_{60}-band) to the periosteum to determine differences in periosteal resorption between the groups, and from the endosteal OTC-band deposited at age 75 days (E_{75}-band) to the endosteum to determine the endosteal appositional growth during age 75–95 days in the different groups. The present investigation also compared normal animals with hypophysectomized animals, using the E_{60}-E_{75} distance as reference distance as described previously[15].

Results. Bone remodelling during age 60–75 days. During the period with no hormone administration, i.e. age 60–75 days, the endosteal growth was the same in the hypophysectomized animals and in the normal animals at the respective section levels along the diaphysis, as earlier shown[15]. Periosteally, the normal animals showed a low appositional growth proximally in the diaphysis and a slight resorption distally. The hypophysectomized animals showed a marked periosteal resorption along the whole of the diaphysis and the cortical width was decreased to about 80% of the normal.

Bone remodelling during age 75–95 days - effect of α-MSH and ACTH (table). Compared with normal controls, the hypophysectomized controls showed a marked periosteal resorption and a marked increase in endosteal growth. This resulted in a total cortical width which was about 80% of that in normal animals at all section levels. Administration of α-MSH markedly decreased the periosteal resorption along the diaphysis compared with the hypophysectomized controls. The decrease showed no marked differences between the different dose groups. Endosteally, there were no differences between hormone-treated animals and hypophysectomized controls. This resulted in an increase in total cortical width in the MSH-treated groups compared with the hypophysectomized controls. α-MSH administration to hypophysectomized animals, compared with normal controls, turned the remodelling towards normal without normalization of the remodelling process, as the periosteal resorption never changed to normal apposition. ACTH-administration to hypophysectomized animals had no effect on the periosteal resorption but slightly decreased the endosteal apposition. There was no or only slight changes in total cortical width. Thus ACTH only slightly affected the remodelling in the hypophysectomized animals.

Discussion. Hypophysectomy causes a significantly increased periosteal bone resorption and an increased endosteal appositional growth in the ventrolateral part of the cortical circumference of the femur in rat. Both periosteal resorption and endosteal apposition are greater distally than proximally in the diaphysis. The same mode of growth

Cortical bone remodelling in the ventro-lateral segment along the femur diaphysis in normal, hypophysectomized and hormone-treated hypophysectomized rats. Reference level correponds to endosteal growth during age 60–75 days. E_{60}-periosteum indicates cortical width measured from the OTC-band deposited at age 60 days to the periosteum. E_{75}-endosteum indicates endosteal growth after age 75 days. Total indicates total cortical width the day the animals were killed

Interval	Group and dose per day and animal	Administration period (days)	Number of animals	Reference level (μm) 32	40	48	56
E_{60}-periosteum	Normals		12	486 (16)	461 (16)	440 (25)	431 (17)
normal controls	Hypophysectomized controls		20	322± 9 (23)	286± 6 (29)	282± 5 (32)	260± 9 (19)
	MSH 1 μg	20	6	342±14 (3)[d]	328±15 (8)[b]	340±21 (4)[b]	320±22 (4)[c]
	MSH 20 μg	20	6	347±12 (7)[d]	342± 9 (10)[a]	298±22 (5)[d]	./.
	MSH 10 μg	10	6	363± 7 (9)[b]	331± 6 (14)[a]	314± 6 (6)[b]	306± 4 (9)[b]
	MSH 20 μg	10	5	361±10 (11)[c]	347± 8 (7)[a]	334± 7 (6)[a]	298± 7 (9)[c]
	ACTH 2 IU/kg	10	6	319±10 (8)[d]	292±10 (13)[d]	260±12 (13)[d]	267±10 (11)[d]
E_{75}-endosteum	Normals		12	27± 4 (16)	28± 4 (16)	34± 5 (25)	39± 6 (17)
normal controls	Hypophysectomized controls		20	80± 3 (23)	84± 3 (29)	86± 3 (32)	98± 6 (19)
	MSH 1 μg	20	6	80± 8 (3)[d]	95±10 (8)[d]	88± 6 (4)[d]	80±13 (4)[d]
	MSH 20 μg	20	6	83± 5 (7)[d]	82± 3 (10)[d]	83± 2 (5)[d]	./.
	MSH 10 μg	10	6	80± 3 (9)[d]	85± 3 (14)[d]	88± 3 (6)[d]	98± 6 (9)[d]
	MSH 20 μg	10	5	86± 3 (11)[d]	83± 8 (7)[d]	84± 5 (6)[d]	90± 2 (9)[d]
	ACTH 2 IU/kg	10	6	62± 6 (8)[c]	76± 3 (13)[d]	88± 3 (13)[d]	93± 6 (11)[d]
Total	Normals		12	545±13 (16)	529± 6 (16)	522±10 (25)	525±10 (17)
normal controls	Hypophysectomized controls		20	416±12 (10)	418± 5 (21)	402±11 (9)	426± 9 (8)
	MSH 1 μg	20	6	462±10 (3)[d]	463±17 (8)[b]	476±17 (4)[b]	456±14 (4)[d]
	MSH 20 μg	20	6	463±10 (7)[c]	463± 8 (10)[a]	429±10 (5)[d]	./.
	MSH 10 μg	10	6	475± 6 (9)[b]	456± 6 (14)[a]	450± 5 (6)[c]	461± 8 (9)[b]
	MSH 20 μg	10	5	478± 9 (11)[b]	471± 8 (7)[a]	466± 4 (6)[a]	443± 7 (9)[c]
	ACTH 2 IU/kg	10	6	413± 9 (8)[d]	407±10 (13)[d]	396±10 (8)[d]	416± 9 (11)[d]

Number of sections in brackets. ./. indicates 2 sections or less.
Values mean±SEM in μm. No SEM-value in normal animals indicates that resorption is present in one or more of the sections. Statistical analysis (Student's t-test) of difference between hypophysectomized controls and hormone-treated groups.

[a] $p<0.1\%$, [b] $0.1\%<p<1\%$, [c] $1\%<p<5\%$, [d] $p>5\%$.

and resorption is found in the medial part of the cortical bone circumference, whereas the remodelling in the posterior part shows a different mode of growth[10,15]. In this investigation, bone remodelling was determined in the ventro-lateral part of the cortical bone circumference.

To increase the survival at hypophysectomy, cortisone acetate was given in a single low dose at age 60 days. As the experimental period began at age 75 days, the effect of cortisone is negligible[16].

Administration of α-MSH to hypophysectomized animals reduced the periosteal resorption but had no effect on the endosteal apposition. Thus the total cortical width increased compared with the hypophysectomized controls. The effect of α-MSH is similar to that of growth hormone (GH) administered under the same experimental conditions[17]. It was earlier proposed that α-MSH stimulates GH-release in adults[6,8,18] and in children[5]. In the present investigation, the effect of α-MSH on the periosteal resorption could not be an effect via GH, as the animals were hypophysectomized. Although the effects of α-MSH and of GH were similar on cortical bone remodelling, the two hormones have no resemblance in aminoacid sequence.

Although different doses of α-MSH were given during 10 and also 20 days, the effect on the periosteal resorption was about the same. This might indicate that the threshold dose to decrease the periosteal resorption is about 1 μg/day or less. Further investigations are needed to determine the lowest dose for effect on periosteal resorption.

Several investigators have suggested that MSH may have adrenocorticotrophic activity[5,6], although the results in other investigations are conflicting[7–9,19]. The present investigation showed that α-MSH in the doses used did not have the same effect as administration of 2 IU/kg and day of ACTH on cortical bone. The effect of α-MSH was on the periosteum and that of ACTH on the endosteum. It is thus not possible at present to determine whether the effect of α-MSH on cortical bone is an effect of α-MSH alone or whether other factors are involved.

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