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Identification and characterization of somatostatin receptors in neonatal rat long bones

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Somatostatin (somatotropin release inhibiting factor; SRIF) has widespread functions as a modulator of neural activity as well as of endocrine and exocrine secretion. In the present paper, the binding characteristics of somatostatin receptors have been investigated in rat long bones using the stable analogue, ^{125}I-SDZ 204-090, as a ligand. Binding studies revealed the presence of a single class of high-affinity binding sites for ^{125}I-SDZ 204-090 on cells prepared from neonatal rat long bones with an equilibrium dissociation constant (K_D) of 70.1 ± 8.2 pM ($n = 3$). An excellent correlation was found between the ability of various somatostatin analogues to inhibit growth hormone in pituitary cells and to displace the binding of ^{125}I-SDZ 204-090 to the bone cell preparation, indicating that the receptors are very similar, if not identical. The localization of the somatostatin-binding sites was examined by autoradiography after labelling *in vitro* and *in vivo*. The binding sites were shown by both procedures to be selectively localized to the metaphysis of rat long bones. The labelling experiments *in vivo* indicate that these receptors can be reached in the living animal by circulating somatostatin analogues. In addition, the analogue SMS 201-995 inhibited the forskolin-stimulated adenylate cyclase activity in bone cell suspensions. These results suggest that somatostatin could be an important regulatory factor in bone metabolism.

INTRODUCTION

Somatostatin (somatotropin release inhibiting factor; SRIF), a tetradecapeptide originally isolated from hypothalamus on the basis of its ability to inhibit growth hormone (GH) release from anterior pituitary cells [1], has been shown to be present in several other tissues [2–4]. Thus somatostatin appears to have widespread functions as a modulator of neural activity as well as of endocrine and exocrine secretion. Inhibitory effects of this peptide on the release of a variety of hormones such as GH, prolactin, glucagon, insulin, gastrin and thyroid-stimulating hormones have been described [4*a*–7]. The regulatory functions of somatostatin are mediated by specific membrane receptors. High-affinity saturable binding sites have been demonstrated in pituitary gland, brain, pancreas and adrenal cortex, where somatostatin appears to play an important role as a physiological regulator [8–11]. There is also some evidence that bone may be a target tissue for somatostatin. The proliferation and differentiation of bone precursor cells and cartilage cells are inhibited by somatostatin *in vitro* [12,13] and somatostatin infusions inhibit the stimulatory effect of testosterone on endosteal bone formation in the mouse [14]. Thus somatostatin may be directly involved in the regulation of bone formation via specific somatostatin receptors, independently of indirect effects resulting from the inhibition of GH release in pituitary cells.

In the present study, we describe the characteristics of the high-affinity binding of the proteolytically stable somatostatin analogue ^{125}I-SDZ 204-090

[(D)Phe-Cys-Tyr-(D)Trp-Lys-Thr-Cys-Thr-ol] [15]

to a preparation of cells derived from 5-day-old rat long bones. The bone cell suspension was prepared from the region including metaphysis and adjacent cartilage and was thus a heterogeneous cell population including all the cell types to be found in this region. This cell suspension will be referred to as 'bone cells' throughout the paper. In addition, we show the autoradiographic localization of these binding sites at the light microscopic level as revealed using this ligand.

EXPERIMENTAL

Materials

Culture media and sera were obtained from Gibco, Basel, Switzerland. The peptides used were obtained as follows: the metabolically stable somatostatin analogue SMS 201-995 (Sandostatin) [(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol], [16] the [Tyr^3]somatostatin derivative SDZ 204-090 and the other somatostatin analogues mentioned were synthesized by W. Bauer at Sandoz Ltd., Basel, Switzerland. Na^{125}I was purchased from Amersham. Chloramine-T was from Fluka, aprotinin was obtained from Pentapharm, and bacitracin was from Serva, Heidelberg, Germany. All other substances were obtained from commercial sources.

Abbreviations used: GH, growth hormone; sCT, salmon calcitonin.

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Preparation of bone cell fractions

Bone cell suspensions were prepared from neonatal rat humerus, radius, ulna, femur, tibia and fibula as previously described [14]. Briefly, the long bones of 5-day-old Sprague–Dawley rats were dissected from the front and hind legs and cleaned of soft tissue. For further discrimination of bone-tissue-derived and bone-marrow-derived cells, the isolated long bones were cut across the diaphysis and the bone marrow was washed out. From each end of each long bone the metaphysis with some epiphysis attached was dissected out and used for preparation of bone cell suspensions. The pieces of bone were minced in Hepes/BSS (10 mM-Hepes, 130 mM-choline chloride, 5 mM-$MgCl_2$, 2.5 mM-$CaCl_2$, 10 mM-glucose, 1 g of bovine serum albumin/100 ml, 10 μg of bacitracin/ml, 300 units of aprotinin/ml and 2 μM-phenylmethane sulphonyl fluoride, pH 7.4) then sucked up and expelled through a plastic pipette continuously for 5 min. Fragments of bone were allowed to settle and discarded. The resulting bone cell suspension was thus a heterogeneous cell population including all the various cell types to be found in the metaphysis and adjacent region. This crude bone-tissue-derived cell suspension will be referred to as 'bone cells' throughout the paper.

Preparation and purification of labelled [Tyr^3]SMS 201-995

Because the stable somatostatin analogue SMS 201-995 contains no tyrosine residues, [Tyr^3]SMS 201-995 (SDZ 204-090) was used for iodination. The biological potency of this analogue is comparable with that of SMS 201-995 as it has the same affinity *in vitro* for the somatostatin recognition site and was found to inhibit GH secretion in similar concentrations to the parent compound [15]. SDZ 204-090 was iodinated by the chloramine-T iodination procedure and purified using h.p.l.c. according to Reubi *et al.* [18]. The iodinated SDZ 204-090 eluted as a single peak, distinct from non-iodinated peptide, using an acetonitrile gradient. The specific radioactivity of the purified ^{125}I-SDZ 204-090 was approx. 2000 Ci/mmol. The labelled compound can be stored at 4 °C for up to 6 weeks as indicated by the fact that no loss of specific binding or increase in non-specific binding is observed.

Radioligand binding studies

The radioligand binding studies were performed with a freshly prepared bone cell fraction. In preliminary experiments, several binding conditions were examined and the following procedure was found to be optimal. Briefly, the bone cell fraction derived from rat long bones was diluted to approx. 1.2×10^7 cells/ml in Hepes/BSS. Binding assays consisted of 70 μl of radioligand, 30 μl of buffer or drug and 200 μl of cell suspension. The experiments were started by addition of the cells to siliconized glass tubes containing radioligand and drug; the tubes were then incubated at room temperature for 120 min. The incubation was stopped by rapid filtration and washing with ice-cold 10 mM-Tris/150 mM-NaCl buffer (4 × 5 ml) over Whatman GF/B glass-fibre filters on a Brandel MR 24 cell harvester. Filters were dried under vacuum, added to vials and radioactivity was counted in an LKB γ-counter at 78 % counting efficiency. Non-specific binding was defined in the presence of 1 μM-$SRIF_{14}$ (Ala-Gly-Lys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Lys) or SMS 201-995. Competition experiments were performed with seven to ten concentrations of drug and 60–90 pM-^{125}I-SDZ 204-090. Saturation experiments were performed with eight concentrations of radioligand ranging from 2 pM to approx. 800 pM. Experiments were carried out in triplicate. Results are expressed as means ± S.E.M. of n independent experiments.

Autoradiographic studies

Previously described autoradiographic procedures were used [19,20]. Briefly, frozen tissue blocks were mounted on microtome chucks and sectioned at −20 °C using a microtome cryostat (Leitz 1720, from Leitz, Wetzlar, Germany) and mounted on gelatin-coated glass slides. Somatostatin receptors were labelled according to the following procedure. Tissue sections were preincubated in Tris/HCl buffer (15 mM, pH 7.4) containing $CaCl_2$ (2 mM) and KCl (5 mM) for 10 min at room temperature and washed twice for 2 min in the same buffer without salts. Incubation was carried out at room temperature for 2 h in Tris/HCl buffer (0.17 M, pH 7.4) containing 1 % bovine serum albumin, bacitracin (40 μg/ml) and $MgCl_2$ (5 mM) to inhibit endogenous proteinases. The ligand concentration was 60 pM. Non-specific binding was determined by using an excess of SMS 201-995 (1 μM). At the end of the incubation, the sections were washed twice for 5 min each in cold incubation buffer containing 0.25 % bovine serum albumin and a final dip in cold double-distilled water before drying the sections under a stream of cold dry air.

Autoradiograms were generated by exposing the labelled sections to [^{3}H-sensitive Ultrofilm (LKB, Broma, Sweden). The exposure time was 10 days. Plastic ^{125}I-labelled standards (Amersham) were exposed along with the labelled tissues. Autoradiograms were analysed using a computer-assisted image analysis system (MCID Imaging Res., Ontario, Canada).

Autoradiography *in vivo* was performed as previously described [9]. The rats (Sprague–Dawley, 5 days old) were anaesthetized with pentobarbital sodium (0.3 mg/10 g intraperitoneal) 15 min prior to application of the ^{125}I-SDZ 204-090 subcutaneously (4×10^6 c.p.m./50 μl). At 60 min after drug application, the animal was perfused intracardially with 50 mM-Sorensen's buffer (pH 7.3) containing 2.5 % glutaraldehyde. A rough estimate of bound ligand was obtained by counting the radioactivity in isolated long bones in a γ-counter. Tissue sections (10 μm thick) were prepared using a microtome cryostat, mounted on gelatin-coated slides and directly apposed to ^{3}H-sensitive Ultrofilms to generate autoradiograms. Exposure time was 6–20 days.

Determination of adenylate cyclase activity

The bone cell suspension was prepared from rat long bones as described. Adenylate cyclase activity was assayed in Medium 199 containing 5 % fetal calf serum, 1 % bovine serum albumin, 2 mM-Ca^{2+} and 0.5 mM-isobutylmethylxanthine in a total volume of 500 μl of cell suspension (1.4×10^7 cells/ml). The reaction was initiated by incubating the cells at 37 °C and adding drugs. The experiment was terminated 5 min later by heating the mixture to 95 °C. Cyclic AMP content was determined by radioimmunoassay [21,22].

Data analysis

Competition data were analysed using the non-linear

regression computer program SCTFIT developed by De Lean [23]. The displacement curves were analysed according to a one-site model. Saturation experiments were calculated with SCTFIT and according to Scatchard [24].

RESULTS

Characterization of ^{125}I-SDZ 204-090 binding sites

Binding experiments were performed with bone cells derived from neonatal rat long bones as described in the Materials and methods section. To further discriminate between bone-tissue-derived and bone-marrow-derived cells, the bones were cut across the diaphysis to wash out the bone marrow. Fig. 1 illustrates the binding of ^{125}I-SDZ 204-090 to bone-tissue-derived and bone-marrow-derived cells. Binding displaceable by somatostatin and SMS 201-995 was only observed in the bone cell fraction, whereas background levels were found in bone marrow cells. Total binding as well as non-specific binding was rather low in the bone marrow cell fraction (less than 1000 c.p.m.) (Fig. 1). Whereas the bone cell fraction revealed a typical displacement of ^{125}I-SDZ 204-090 label by SMS 201-995 ($IC_{50} = 8.9 \times 10^{-10}$ mol/l), no displaceable binding could be detected in the bone marrow-derived cell suspension. We thus subsequently used the freshly prepared bone cell preparation to further characterize the putative somatostatin recognition sites in rat long bone tissue.

Kinetic experiments

A representative time course of specific ^{125}I-SDZ 204-090 binding to bone cells at room temperature is shown in Fig. 2. The non-specific binding, determined in the presence of 1 μM-SMS 201-995, was less than 20% of total binding. The amount of ^{125}I-SDZ 204-090 specifically bound increased with time, reaching a plateau value within 120 min, and then remained stable up to 240 min at room temperature. Therefore in all routine binding experiments performed to characterize the ^{125}I-SDZ 204-090 binding sites in rat long bones, 120 min incubation at room temperature were selected as optimal binding conditions.

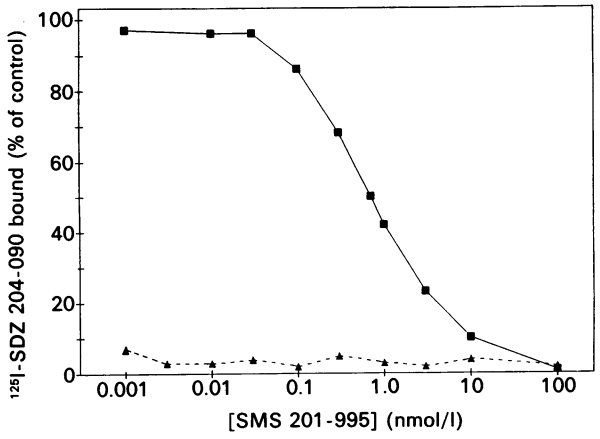

Fig. 1. Binding of ^{125}I-SDZ 204-090 to bone-tissue-derived and bone-marrow-derived cells

Binding of ^{125}I-SDZ 204-090 was measured by incubating the cells at room temperature for 120 min in the presence of various concentrations of unlabelled ligand. Specifically bound ^{125}I-SDZ 204-090, i.e. total minus non-specific binding, is plotted as a function of unlabelled SMS 201-995 concn. for bone cells (■) and bone marrow cells (▲). The maximal specific binding to bone-tissue-derived cells in the absence of competitor was taken as 100% (control). Total and non-specific binding was rather low in the bone marrow cell fraction (less than 1000 c.p.m.). Values are the means of three determinations.

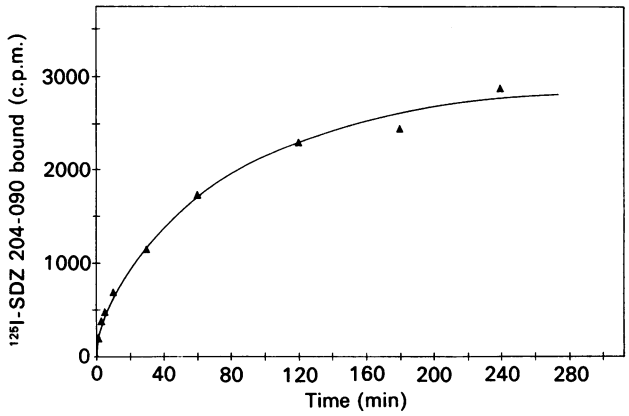

Fig. 2. Time course of binding of ^{125}I-SDZ 204-090 in bone cell suspensions

The bone cells were incubated with ^{125}I-SDZ 204-090 for the indicated times at room temperature, and the cell-bound radioactivity was counted. Specific binding was calculated by subtracting non-specific binding from total binding. The non-specific binding, as determined in the presence of 1 μM-SMS 201-995, was less than 20% of total binding for the indicated times. Values are expressed as c.p.m. of specific binding, and each point represents the mean of three determinations.

Saturation experiments

The concentration-dependence of ^{125}I-SDZ 204-090 binding is shown in Fig. 3. Specific binding was determined after incubating the cells with increasing concentrations of ^{125}I-SDZ 204-090 at room temperature for 2 h. ^{125}I-SDZ 204-090 exhibited high-affinity binding sites in the sub-nanomolar range. Computer fitting of the data by SCTFIT [23] revealed that the binding exhibited the characteristics expected for the binding of homogeneous radiolabelled peptide to a single class of non-interacting binding sites. Scatchard [24] transformations of the saturation curves were linear, demonstrating the presence of a single class of high-affinity binding sites for somatostatin in rat long bones (Fig. 3, inset). In this experiment, the apparent equilibrium dissociation constant K_D was 61.1 pM and the receptor number (R_t) was 200 binding sites/cell. This value is clearly underestimated since we used a heterogeneous cell population including all the cell types to be found in the metaphysis and adjacent region. In contrast, the autoradiographic studies revealed specific somatostatin binding sites in a very restricted region of the metaphysis of neonatal rat long bones. Cells localized in this region must, therefore, possess many more than 200 binding sites/cell. The pK_D values agreed closely in three independent saturation experiments with different preparations of cells and tracer ($K_D = 70.1 \pm 8.2$ pM).

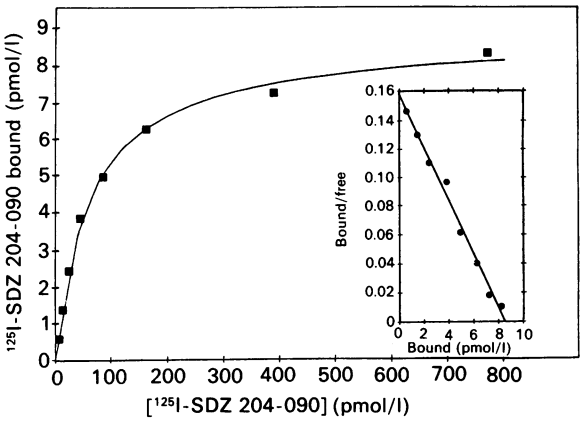

Fig. 3. Concentration-dependence of ^{125}I-SDZ 204-090 binding to bone cell suspensions

Bone cells (1.2×10^7 cells/ml) were incubated in Hepes/BSS (pH 7.4) containing the indicated concentration of ^{125}I-SDZ 204-090 in the absence and presence of 1 μM-SMS 201-995. After 2 h at room temperature, saturable ^{125}I-SDZ 204-090 binding was determined as described in the Experimental section. Inset: binding data plotted by the method of Scatchard [24]. The maximum binding capacity (B_{max}) was calculated from the intercept on the abscissa and the affinity (K_D) from the slope of the line.

Competition experiments

To further characterize the structural requirements of somatostatin binding to rat long bones, we determined the inhibitory effect of some somatostatin analogues in competition experiments using ^{125}I-SDZ 204-090 as ligand. The peptides used differed in chain length and in selective amino acid substitutions. All compounds tested inhibited the binding of ^{125}I-SDZ 204-090 to the same levels of non-specific binding with different affinities in a monophasic manner. The order of activity was consistent with their ability to inhibit GH release from pituitary cells. The analogues SDZ 203-304 [Lys-ol^8]SMS and SDZ 204-354 [Orn^5]SMS, which are relatively ineffective in inhibiting GH release from cultured rat pituitary cells, also exhibited weak affinities to the ^{125}I-SDZ 204-090 recognition site of bone cells. In contrast, $SRIF_{14}$ and SMS 201-995 showed about two orders of magnitude higher affinities than these analogues. Representative competition curves obtained with the various somatostatin analogues and salmon calcitonin (sCT) are shown in Fig. 4. An excellent correlation was found between the ability of these peptides to inhibit GH release from pituitary cells *in vitro* and to displace the binding of ^{125}I-SDZ 204-090 to bone cells ($r = 0.9935$; $P < 0.001$). Therefore the pharmacological profiles of the somatostatin receptor in these various tissues seem to be very similar.

sCT up to 1 μM did not affect the specific binding of ^{125}I-SDZ 204-090 to bone cells (Fig. 4). The inability of sCT to displace ^{125}I-SDZ 204-090 binding demonstrates that ligand binding to bone cells does not interfere with CT recognition sites.

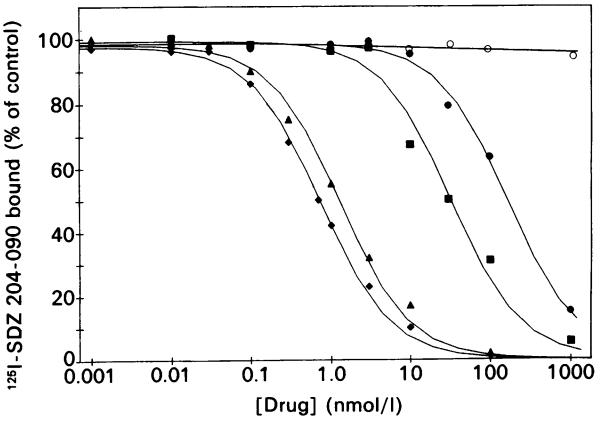

Fig. 4. Concentration-dependence for the inhibition of ^{125}I-SDZ 204-090 binding by somatostatin analogue and calcitonin

Bone cells (1.2×10^7 cells/ml) were incubated at room temperature with ^{125}I-SDZ 204-090 (70000 c.p.m./tube) and the indicated concentration of SMS 201-995 (◆), $SRIF_{14}$ (▲), [Lys-ol^8]SMS (203-304) (●), [Orn^5]SMS (204-354] (■), or sCT (○). After 2 h the amount of bound ^{125}I-SDZ 204-090 was determined as described in the Experimental section. Results are expressed as a percentage of the maximal specific binding to bone cells in the absence of competitor. In all experiments the non-specific binding was less than 20% of the total binding (approx. 3600 c.p.m.). The data are the mean values of three determinations and the lines represent computer-fitted regression lines determined as described in the Experimental section.

Localization of specific ^{125}I-SDZ 204-090 binding sites in rat long bones by autoradiography *in vitro* and *in vivo*

Somatostatin binding sites were revealed autoradiographically by incubating mounted microtome tissue sections with the labelled ligand ^{125}I-SDZ 204-090. Under the conditions used in these experiments, the binding of ^{125}I-SDZ 204-090 has been shown in several different tissues to present the characteristics expected of somatostatin receptors [9,19,20]. Fig. 5 illustrates, at different levels of resolution, the localization of specific ^{125}I-SDZ 204-090 binding sites to long bone tissue sections. As can be seen by comparing Figs. 5(*a*) and 5(*e*), the zone showing a high density of binding sites was confined to the metaphysis of the rat long bones. However, from these autoradiographic studies it is not clear which cell types do have specific somatostatin binding sites. Much lower binding was observed in the diaphysis and epiphysis as well as in the skin (Figs. 5*a* and 5*b*). Only the binding to the metaphysis was displaced by co-incubation with 1 μM unlabelled SMS 201-995, while binding to the other bone areas and to the skin was non-specific (Fig. 5*d*).

Autoradiography *in vivo* in tissues from animals injected with 4×10^6 c.p.m. of ^{125}I-SDZ 204-090 at 60 min before death showed a distribution of specific binding identical to that seen in the experiments *in vitro* (Fig. 5*c*). Pretreatment of another group of animals with unlabelled SMS 201-995 (100 μg/50 μl) at 45 min before injection of the tracer did not reveal binding of ^{125}I-SDZ 204-090 to bones. These results indicate that the specific somatostatin receptors in bone tissues interact with circulating SMS 201-995.

Inhibition of forskolin-stimulated adenylate cyclase activity by SMS 201-995

Basal cyclic AMP levels in the bone cell suspension

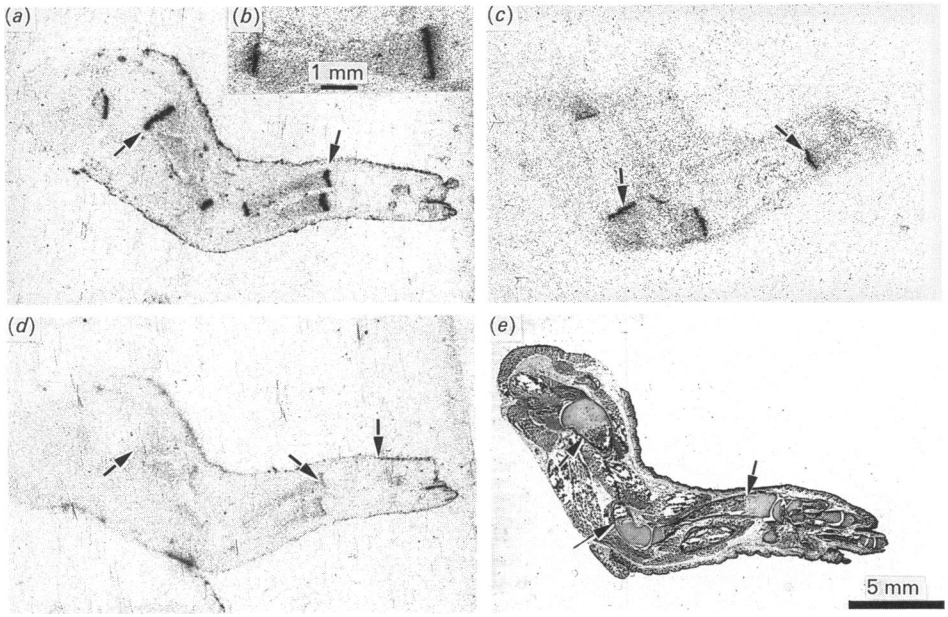

Fig. 5. Light-microscopic autoradiograms showing the localization of ^{125}I-SDZ 204-090 binding sites in the metaphysis of rat long bones

(*a*) Photograph from a film autoradiogram after labelling of somatostatin receptors *in vitro*. Dark areas indicate zones of high density of binding sites. Note the high density of sites localized in the metaphysis of the long bones (arrows). This is illustrated at a higher level of magnification in (*b*). (*c*) Autoradiogram obtained after labelling of receptors *in vivo*. The distribution obtained is similar to that obtained *in vitro*. (*d*) Distribution of non-specific (non-displaceable) binding. Note the complete blockade of binding to the bones but not to the skin (arrows). (*e*) Tissue section stained with eosin/hematoxylin. The metaphyses are indicated by arrows.

were 2.7 ± 0.3 pmol of cyclic AMP/tube ($n = 8$). SMS 201-995 at 1 μM did not significantly affect these basal cyclic AMP levels. Forskolin (0.1 μM–0.1 mM) stimulated adenylate cyclase activity in a concentration-dependent manner (results not shown). It was decided to use 10 μM-forskolin, a concentration that increased cyclic AMP levels by approx. 10-fold (26.3 ± 0.7 pmol/tube), to seek evidence for an inhibitory effect of somatostatin. SMS 201-995 at 1 μM inhibited significantly the forskolin-dependent increase in cylcic AMP levels ($P < 0.01$; one-tailed Student's *t*-test). An inhibitory effect of $29.5 \pm 2\%$ could be observed by treatment of bone cells with SMS 201-995 ($n = 8$).

DISCUSSION

The main findings presented in this paper can be summarized as follows. 1. Specific and high-affinity binding sites for ^{125}I-SDZ 204-090 have been characterized on cells prepared from long bones of 5-day-old rats. 2. These specific binding sites appear to be comparable in their pharmacological specificity to those already observed in the pituitary, since for several analogues there is an excellent correlation between the ability to displace the binding of ^{125}I-SDZ 204-090 to bone cells and their biological action in inhibiting GH release from pituitary cells. 3. Some evidence for a functional link of these specific binding sites to an intracellular second messenger system is provided by the ability of SMS 201-995 to inhibit the forskolin-dependent increase in cyclic AMP levels in our bone cell fraction as has been reported for somatostatin in many other target tissues [24,25]. 4. Selective localization of the ^{125}I-SDZ 204-090 binding sites in the metaphysis of rat long bones was shown by comparing autoradiography *in vitro* and hematoxylin/eosin-stained sections. 5. That these binding sites may be accessible to circulating hormone was demonstrated by specific labelling of these receptors *in vivo*, where circulating ^{125}I-SDZ 204-090 interacts with the somatostatin-binding sites in rat long bones. Thus we were able to demonstrate for the first time the presence of specific high-affinity binding sites for somatostatin in the rat long bone metaphysis which may be important in the regulation of bone metabolism.

Previous studies have suggested a modulatory role for somatostatin in bone [12,14,25]. However, these studies have generally not supported a direct action of the peptide on bone growth. Up to now, only indirect evidence has been presented. Weiss *et al.* [12] demonstrated the inhibitory effect of somatostatin on the proliferation and differentiation of cartilage and bone precursor cells *in vivo*. Local injections of somatostatin resulted in a 75% reduction in cell proliferation. In addition, osteogenesis was impaired after local injection of somatostatin and these effects occurred without any apparent systemic alterations, indicating a local effect of somatostatin. Moreover, recent experiments reported by Marie *et al.* [14] have suggested that somatostatin is involved in growth regulation in rat long bones. It was demonstrated that testosterone-induced bone formation could be decreased by somatostatin and SMS 201-995. Although the inhibitory effect of somatostatin and SMS 201-995 on endosteal bone formation could be observed in the mouse, no marked decreases in serum GH-levels were measured under these experimental conditions. Therefore, a factor other than reduction of circulating GH-levels may have contributed to decreased bone formation.

An interesting clinical correlation of these findings has recently been presented by Tauber *et al* [26]. Treatment of 10 tall adolescent children with Sandostatin (SMS 201-995) resulted in a significant reduction in growth rate after 6 months of treatment in nine of the patients. Moreover, acceleration of bone maturation on SMS 201-995 therapy occurred in seven patients.

Thus long bones may be a target organ for somatostatin. We therefore questioned whether somatostatin may contribute locally to the regulation of bone growth and metabolism in addition to systemic hormonal effects via GH and/or insulin-like growth factor 1, which do have direct growth-promoting effects on cartilage *in vivo* [27]. The present results demonstrate the occurrence of specific somatostatin binding sites in bone tissue. Labelling experiments *in vivo* clearly show that specific somatostatin-binding sites were confined to the metaphysis of neonatal rat long bones as could be demonstrated by autoradiography *in vitro* and that these specific hormone-binding sites are accessible to a circulating analogue of somatostatin. This again indicates that bone tissue might be a direct target organ for somatostatin, allowing it to function as a local regulatory factor in bone. However, from the experiments presented in this paper, it is not clear which cell type does have specific somatostatin-binding sites. The region identified by autoradiography includes osteoblasts, osteoclasts, cartilage cells and endothelial cells from blood vessels.

Although the present data indicate that bone tissue may be a target organ for somatostatin, the physiological significance of this has yet to be proven. Nothing is known about the mechanism of somatostatin action on bone tissue. The cyclic AMP content in the bone cell preparation was selected as one relevant parameter to show the linkage of these binding sites to signal transduction mechanisms. Changes in cyclic AMP content induced by somatostatin have been reported previously for various tissues which are likely to be target organs for this hormone, such as pituitary cells [28], pancreatic islets [29], intestinal cells [30] and liver cells [31]. Somatostatin has been reported to inhibit the forskolin-induced cyclic AMP increase in most, if not all, target cells [32]. In agreement with these results, we demonstrated an inhibitory effect of SMS 201-995 on the forskolin-induced cyclic AMP increase in bone cell suspensions. However, only a part of the cells prepared in our bone cell suspensions are somatostatin receptor positive. Since forskolin stimulates the adenylate cyclase directly without cell specificity, no specific inhibitory effect for somatostatin can be expected. This may account for the fact that inhibition of forskolin-stimulated cyclic AMP production by somatostatin required a much higher concentration of the peptide than was needed for saturation of the receptor.

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