Adrenomedullin is a potent stimulator of osteoblastic activity in vitro and in vivo

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Adrenomedullin is a potent stimulator of osteoblastic activity in vitro and in vivo. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E1113–E1120, 1997.—Adrenomedullin is a 52-amino acid vasodilator peptide produced in many tissues, including bone. It has 20% sequence identity with amylin, a regulator of osteoblast growth, and circulates in picomolar concentrations. The present study assesses whether adrenomedullin also acts on osteoblasts. At concentrations of 10–12 M and greater, adrenomedullin produced a dose-dependent increase in cell number and [3H]thymidine incorporation in cultures of fetal rat osteoblasts. This effect was also seen with adrenomedullin-(15–52), -(22–52), and -(27–52), but adrenomedullin-(40–52) was inactive. These effects were lost in the presence of amylin blockers, suggesting they were mediated by the amylin receptor. Adrenomedullin also increased [3H]thymidine incorporation into cultured neonatal mouse calvaria but, unlike amylin, did not reduce bone resorption in this model. Adrenomedullin stimulated phenylalanine incorporation into both isolated osteoblasts and calvaria. When injected daily for 5 days over the calvariae of adult mice, it increased indexes of bone formation two- to threefold (P < 0.001) and increased mineralized bone area by 14% (P = 0.004). It is concluded that adrenomedullin regulates osteoblast function and that it increases bone mass in vivo. The potential of this family of peptides in the therapy of osteoporosis should be further evaluated.

Amylin; calvaria; bone resorption; peptide fragments

Adrenomedullin is a 52-amino acid peptide first described in 1993 by Kitamura et al. (20) and recently reviewed by Kitamura and Eto (18). It was originally identified in a human pheochromocytoma and has since been found to be present in normal adrenal medulla and in many other tissues, including the atria, ventricles, endothelial cells, lungs, brain, kidneys, and bone (14, 23). It circulates in picomolar concentrations in both rats and humans (19). It is a potent vasodilator, acting directly on the renal, cerebral, mesenteric, pulmonary, and systemic circulations, including the vascular supply of the skeleton (17). Its hemodynamic effects are probably mediated via receptors on vascular smooth muscle cells and possibly endothelial cells. The kidney may also be a significant target tissue. Binding to renal tubular membranes has been observed, and sodium, potassium, and water excretion are increased by adrenomedullin. It is a bronchodilator, and it modulates release of pituitary and vasoactive hormones. The adrenomedullin receptor has recently been cloned (16). It contains seven transmembrane domains and couples to adenylyl cyclase. It has a structural resemblance to the other G protein-linked receptors, and expression of the receptor mRNA is widespread.

Adrenomedullin shows ~20% sequence identity with amylin and calcitonin gene-related peptide and slightly less with calcitonin itself (24). All these peptides have an NH2-terminal ring created by a disulfide bond and are amidated at their COOH terminals. Adrenomedullin differs from the others in that it has a linear NH2-terminal extension, consisting of 15 amino acids in the human and 13 in the rat. The other members of this group inhibit bone resorption, probably via a direct action on the osteoclast calcitonin receptor (1, 36). We have recently shown that amylin also has direct effects on osteoblast function, stimulating cell proliferation in vitro and increasing indexes of bone formation in vivo (2, 3). In light of these findings, we have assessed the actions of adrenomedullin in a variety of models to determine whether it too is a modulator of bone cell function.

METHODS

Osteoblast-like cell culture. Osteoblasts were isolated by collagenase digestion from 20-day fetal rat calvaria, as previously described (21). Calvaria were dissected aseptically, and the frontal and parietal bones were stripped of their perios- teum. Only the central portions of the bones, free from suture tissue, were collected. The calvaria were treated twice with phosphate-buffered saline (PBS) containing 3 mM EDTA (pH 7.4) for 15 min at 37°C in a shaking water bath. After being washed once in PBS, the calvaria were treated twice with 3 ml of 1 mg/ml collagenase for 7 min at 37°C. After the supernatants were discarded from digestions I and II, the calvaria were treated an additional two times with 3 ml of 2 mg/ml collagenase (30 min, 37°C). The supernatants of digestions III and IV were pooled and centrifuged, and the cells were washed in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS), suspended in 3 mM EDTA (pH 7.4) for 15 min at 37°C in a shaking water bath. After being washed once in PBS, the cells were rinsed in Eagle's minimum essential medium (MEM) with 10% fetal calf serum (FCS), suspended in additional DMEM-10% FCS, and placed in 75-cm2 flasks. The cells were incubated under 5% CO2-95% air at 37°C. Confluence was reached by 5–6 days, at which time the cells were subcultured. After trypsinization using trypsin-EDTA (0.05%-0.53 mM), the cells were rinsed in Eagle's minimum essential medium (MEM) with 5% FCS, resuspended in fresh medium, and then seeded at 5 × 106 cells/ml in 24-well plates (0.5 ml cell suspension/well, i.e., 1.4 × 104 cells/well). The osteoblast-like character of these cells has been established by demonstration of high levels of alkaline phosphatase activity and osteocalcin production (10) and a sensitive adenylate cyclase response to parathyroid hormone and prostaglandins (12).

Proliferation studies (cell counts and thymidine incorporation) were performed both in actively growing and nonactively growing cell populations. To produce actively growing
cells, subconfluent populations (24 h after subculturing) were changed to fresh MEM, which contained 1% FCS and the experimental compounds. To produce nonactively growing cells, subconfluent populations were changed to serum-free medium with 0.1% bovine serum albumin plus the experimental compounds. Cell numbers were analyzed at 6, 24, and 48 h after addition of the peptide or vehicle. The cell numbers were determined after cells were detached from the wells by exposure to trypsin-EDTA (0.05%/0.53 mM) for ~5 min at 37°C. Counting was performed in a hemocytometer chamber. Results were expressed per well. [3H]thymidine incorporation into actively growing and nonactively growing cells was assessed by pulsing the cells with [3H]thymidine (1 µCi/well) 2 h before the end of the experimental incubation. The experiment was terminated at 6, 24, or 48 h by washing the cells in MEM containing cold thymidine followed by the addition of 10% trichloroacetic acid. The precipitate was determined after cells were detached from the wells by exposure to trypsin-EDTA (0.05%/0.53 mM) for ~5 min at 37°C. Counting was performed in a hemocytometer chamber. Results were expressed as disintegrations per minute (dpm) per well. For both cell counts and thymidine incorporation, each experiment was performed at least four different times using experimental groups consisting of at least six wells. [3H]phenylalanine incorporation was assessed by pulsing the cells with [3H]phenylalanine (1 µCi/well) for 4 h before the end of the experimental incubation.

Bone organ culture. Bone resorption studies were carried out in neonatal mouse calvaria as described previously (31). Mice were injected subcutaneously with 5 µCi 45Ca at 2 days of age, and calvaria were dissected out 4 days later. Calvaria were preincubated for 24 h in medium 199 with 0.1% bovine serum albumin and then transferred to fresh medium containing vehicle or peptide. Incubation was continued for a further 48 h. To assess DNA synthesis, [3H]thymidine (0.6 µCi/ml) was added in the last 4 h of the incubation, as described previously (22). Similarly, in cultures in which protein synthesis was determined, [3H]phenylalanine (1 µCi/ml) was added. There were five to seven calvaria in each group.

In vitro studies. The local effects of adrenomedullin on bone histology in vivo were assessed in adult mice, using the model we have previously described (4). Sexually mature male ARC Swiss Webster mice, aged between 40 and 50 days and weighing 25–35 g, were given injections (25 µl) over the periosteum of the right hemicalvaria for 5 consecutive days. The animals were maintained on a low-calcium diet (0.1%) from 5 days before the first injection. Two groups of mice (n = 12 in each) were injected daily with adrenomedullin in doses of 4 × 10−10 and 4 × 10−9 mol, respectively. These doses were chosen on the basis of our experience with other bone-active peptides in this model. Animals in the control group (n = 16) were injected with vehicle (water) only. All animals were killed 1 wk after the last injection. The study had the approval of the local institutional review board.

The calvaria were dissected free of soft tissue, and gross morphology was assessed by examination of the intact calvaria under a dissection microscope. Bone tissue was fixed in 10% phosphate-buffered Formalin, dehydrated in a graded series of ethanol solutions, and embedded undecalified in methyl methacrylate resin. Sections (4 µm thick) were cut on a Leica rotary microtome (Leica Instruments, Nussloch, Germany) using a tungsten-carbide knife, mounted on gelatin-coated slides, and air-dried. The sections were stained with a Goldner trichrome stain and examined with the use of an Olympus BX 50 microscope (Olympus Optical, Tokyo, Japan), which was attached to an Osteomeasure Image Analyzer (Osteometrics, Atlanta, GA). Histomorphometric analyses were made of three adjacent fields (using a ×20 objective) in each hemicalvarium. This results in measurements being made over ~90% of the length of each hemicalvarium. The parameters assessed are as defined by the American Society for Bone and Mineral Research (27) and are expressed per millimeter of calvarial length. Osteoblasts were defined as cuboidal cells immediately adjacent to osteoid. Osteoclast numbers included only multinucleated cells. The various surface estimates were based on measurements of both periosteal and intramembranous surfaces: those eroded by osteoclasts (eroded perimeter), those immediately adjacent to osteoclasts (osteoclast perimeter), and those immediately adjacent to osteoblasts (osteoblast perimeter). The precisions of these histomorphometric measurements in our laboratory (expressed as coefficients of variation of paired measurements) are as follows: mineralized bone area 1.3%, osteoid area 6.9%, osteoblast perimeter 6.8%, osteoblast number 1.7%, eroded perimeter 6.7%, osteoclast perimeter 7.9%, osteoclast number <1.0%, and calvarial length 0.2%. All measurements were made by one operator (J. Cornish) who was blinded to the treatment group of each bone.

Materials. Human adrenomedullin and its fragments were synthesized on methylbenzyldiamine resin, using standard solid-phase procedures, and cleaved with hydrogen fluoride-anisole. Sequences containing a disulfide bridge were cyclized by titration with I2 in 90% acetic acid-water solutions (26). Crude materials were purified by gel filtration on Sephadex columns in 50% acetic acid followed by gradient elution on C-18 silica using acetonitrile:0.1% trifluoroacetic acid eluants. Homogeneity of final peptides was assessed by thin-layer chromatography, analytic high-performance liquid chromatography, amino acid analysis, and matrix-assisted laser-desorption-ionization mass spectroscopy. Purities were usually >98%.

EDTA and collagenase were obtained from Sigma Chemical, St. Louis, MO. Trypsin-EDTA, MEM, DMEM, medium 199, and FCS were from GIBCO Laboratories, Grand Island, NY. [3H]thymidine, [3H]phenylalanine, and 45Ca chloride were from Amersham, England.

Statistical analysis. Data are presented as means ± SE. The in vitro experiments were analyzed using Student’s t-tests for pair-wise comparisons. Comparisons across more than two groups have used analysis of variance (ANOVA), and subsequent pair-wise comparisons were made using the Bonferroni inequality to adjust for multiple comparisons, where appropriate. A value of α = 0.05 was accepted as significant throughout. Where several experiments have been shown in one figure, data are presented as treatment-to-control ratios, but the P values shown were calculated using the data from the individual experiments before the data were pooled. All other data are from individual, representative experiments.

In the in vivo experiment, the primary end-point for each histomorphometric index was the determination of whether it was different in the injected right hemicalvaria from the contralateral, uninjected bone of the same animal. Ratios of each index in these two bone regions were derived and compared between groups by ANOVA. These data and the associated statistical significances are presented in Figs. 7 and 8. The absolute values of each histomorphometric index are also presented for the injected and uninjected hemicalvaria by treatment group (Table 1).
RESULTS

Effect of adrenomedullin on isolated osteoblasts. The effect of adrenomedullin on proliferation of fetal rat osteoblasts was assessed by the measurement of cell numbers. Treatment with adrenomedullin for 24 h, in cultures grown in medium containing 1% FCS, produced a dose-dependent increase in the numbers of actively growing osteoblasts (Fig. 1). A significant increase was observed at adrenomedullin concentrations of $10^{-12}$ M and greater. This stimulation was maintained for 48 h (Fig. 2A). To determine whether proliferation in response to adrenomedullin was dependent on the basal growth rate of the cells, these experiments were repeated in nonactively growing osteoblastic preparations. The time course of the increase in cell number in response to adrenomedullin ($10^{-10}$ M) in these cells was similar to that seen in actively growing cells (Fig. 2B).

The effect of adrenomedullin on DNA synthesis in osteoblasts was assessed by the measurement of $[^3H]$thymidine incorporation into isolated fetal rat osteoblasts. Treatment with adrenomedullin for 24 h produced a dose-dependent increase in $[^3H]$thymidine incorporation (Fig. 3A). This stimulation was maintained for 48 h (Fig. 3B).

To determine in what part of the adrenomedullin molecule its anabolic activity resides, we investigated the effects of various synthetic fragments of the peptide on the proliferation of fetal rat osteoblasts (Fig. 4). Treatment for 24 h with the (15—52), (22—52), or (27—52) peptides produced a similar stimulation of proliferation to that from the intact adrenomedullin molecule. However, adrenomedullin-(40—52) did not produce any increase in cell number. When the disulfide bond of adrenomedullin was broken, the resultant reduced peptide had diminished agonist activity, which was detectable only at concentrations $\geq 10^{-10}$ M, with a maximal effect one-half of that seen with the intact molecule (data not shown).

In view of the similarity of the present findings to those of amylin in these model systems, we assessed the effects of putative amylin receptor blockers on these

### Table 1. Effects of adrenomedullin on histomorphometric indexes in adult mouse calvaria in vivo

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Adrenomedullin (4 x $10^{-10}$ mol)</th>
<th>Adrenomedullin (4 x $10^{-9}$ mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninjected</td>
<td>Injected</td>
<td>Uninjected</td>
</tr>
<tr>
<td>Osteoid area</td>
<td>0.0025 (0.0004)</td>
<td>0.0021 (0.0003)</td>
<td>0.0016 (0.0003)</td>
</tr>
<tr>
<td>Ob perimeter</td>
<td>0.69 (0.10)</td>
<td>0.76 (0.07)</td>
<td>0.72 (0.10)</td>
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<tr>
<td>Number of Obs</td>
<td>65 (8)</td>
<td>68 (5)</td>
<td>57 (5)</td>
</tr>
<tr>
<td>Eroded Pm</td>
<td>0.74 (0.10)</td>
<td>0.76 (0.10)</td>
<td>0.77 (0.13)</td>
</tr>
<tr>
<td>Oc Pm</td>
<td>0.12 (0.01)</td>
<td>0.13 (0.02)</td>
<td>0.12 (0.02)</td>
</tr>
<tr>
<td>Number of Ocs</td>
<td>4.4 (0.3)</td>
<td>4.5 (0.3)</td>
<td>4.4 (0.4)</td>
</tr>
<tr>
<td>Periosteal area</td>
<td>0.014 (0.002)</td>
<td>0.013 (0.001)</td>
<td>0.013 (0.002)</td>
</tr>
<tr>
<td>Bone area</td>
<td>0.113 (0.005)</td>
<td>0.106 (0.005)</td>
<td>0.115 (0.005)</td>
</tr>
</tbody>
</table>

Data are means with SE in parentheses. Significance of treatment effects are shown in Figs. 7 and 8. Ob, osteoblast; Pm, perimeter; Oc, osteoclast.

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Fig. 1. Dose response of adrenomedullin (ADM) treatment for 24 h on cell no./well in cultures of actively growing fetal rat osteoblasts. Data have been pooled from 2 separate experiments, are expressed as ratios of treatment to control, and are means ± SE. *Significantly different from control.

Fig. 2. Time course of effect of ADM ($10^{-10}$ M) on cell no./well in actively (A) and nonactively (B) growing fetal rat osteoblasts. Data are means ± SE. Significantly different from control: *P < 0.003 and **P < 0.01.
actions of adrenomedullin. Amylin-(8-37) is a specific blocker at the amylin receptor in skeletal muscle (7), which we have recently shown to block the proliferative effect of amylin in fetal rat osteoblasts (J. Cornish, unpublished observations). Amylin in which the disulfide bond has been broken (reduced amylin) is also an effective amylin blocker in bone (J. Cornish, unpublished observations). These peptides completely blocked the proliferative effects of both intact adrenomedullin (Fig. 5) and its (27-52) fragment (data not shown) in these cells. In contrast, reduced adrenomedullin was a less effective antagonist, diminishing stimulation of cell numbers by one-half when present in a 10-fold excess with the intact peptide (data not shown).

The stimulation of osteoblast proliferation by adrenomedullin was accompanied by similar changes in [3H]phenylalanine incorporation into these cells, implying similar changes in protein synthesis. Thus the incorporation of this amino acid was 3,534 ± 664 dpm/well in control cells, 3,815 ± 68 dpm/well in cells exposed to adrenomedullin 10^-10 M, and 3,818 ± 100 dpm/well in cells exposed to adrenomedullin-(27-52) 10^-10 M (P < 0.05 for both compared with control).

Effect of adrenomedullin in bone organ culture. The effects of adrenomedullin were also assessed in cultured neonatal mouse calvaria. There was a dose-dependent increase in [3H]thymidine incorporation (Fig. 6A) consistent with the findings in isolated cells. However, there was no significant change in 45Ca release from prelabeled calvaria treated for 48 h with adrenomedullin at concentrations of 10^-8 to 10^-11 M (Fig. 6B), and 45Ca release stimulated by parathyroid hormone (10^-8 M) was also unaffected by adrenomedullin (data not shown). In contrast, amylin typically reduces basal resorption by 30% in this model (2). Incorporation of [3H]phenylalanine was increased from 27.3 ± 0.7 dpm/µg in control bones to 30.1 ± 0.6 and 30.0 ± 0.6 in bones exposed to adrenomedullin (10^-9 M) and adrenomedullin-(27-52) (10^-9 M), respectively (P < 0.05 for both compared with control).

Effect of adrenomedullin in vivo. Table 1 sets out the histomorphometric indexes in the uninjected and injected hemicalvaria from each of the three groups. The statistical analysis has been performed on the ratios of each index in the injected to the uninjected halves of each calvaria, and these results are shown in Figs. 7 and 8. There were two- to threefold increases in the indexes of osteoblast activity in those bones exposed to either dose of adrenomedullin. Resorption indexes showed slight upward trends in the presence of adrenomedullin, but only for eroded perimeter was this significant. Mineralized bone area was increased by 13.6% with the higher dose of adrenomedullin. Periosteal area was not changed (P = 0.54).

**DISCUSSION**

The present data establish that adrenomedullin acts on osteoblasts, whether they be of fetal rat origin cultured as disaggregated cells, derived from neonatal mice and cultured as an intact tissue, or studied in vivo in adult mice. Stimulation of cell proliferation occurs whether assessed by cell numbers or by thymidine incorporation into DNA, indicating that the result is not dependent on the measurement technique and it is also independent of the basal growth rate of the osteo-

**Fig. 3.** A: dose response of ADM treatment on [3H]thymidine incorporation into nonactively growing fetal rat osteoblasts at 24 h. There is a significant treatment effect when assessed by analysis of variance (ANOVA; P = 0.01). B: time course of effect of ADM on [3H]thymidine incorporation into nonactively growing fetal rat osteoblasts. Data are means ± SE. *Significantly different from control.

**Fig. 4.** Effect of indicated fragments of ADM on cell no./well in cultures of actively growing fetal rat osteoblasts cultured for 24 h. Data from 1 representative experiment for each fragment have been pooled and are expressed as ratios of treatment to control. Data are means ± SE. Significantly different from control: *P < 0.01.

**Fig. 5.** Effect of adrenomedullin on cell no./well in cultures of actively growing fetal rat osteoblasts cultured for 24 h. Data from 1 representative experiment for each fragment have been pooled and are expressed as ratios of treatment to control. Data are means ± SE. Significantly different from control: *P < 0.01.
blasts. The degree of stimulation of osteoblast proliferation by adrenomedullin is comparable to that produced by recognized osteoblast growth factors such as transforming growth factor-β and insulin-like growth factor I, which each increase cell numbers by ~20% in our laboratory. Adrenomedullin is not a nonspecific mitogen, however, because it inhibits proliferation of its principal target, the vascular smooth muscle cell (15). The explanation for such opposing effects in these tissues is unknown, but two of the second messenger systems linked to G protein-coupled receptors [adenosine 3',5'-cyclic monophosphate (cAMP) and diacylglycerol] can have opposing effects on cell proliferation (29). Thus differential activation of these systems could account for such an effect. Testing of this hypothesis must await an assessment of the second messenger systems involved in the action of adrenomedullin on osteoblasts.

Adrenomedullin also increases protein synthesis in vitro and the area of mineralized and unmineralized bone in vivo. The changes in bone area are comparable to those produced by amylin (3) and insulin (6) in this model and are greater than those associated with calcitonin use (3). The histomorphometric changes are unlikely to represent a nonspecific effect of the injected peptide, since other peptides, such as calcitonin gene-related peptide (3) and COOH-terminal parathyroid hormone-related peptide (5), have no effect on osteoblast activity in this model. How the concentrations of adrenomedullin used here compare with those occurring in vivo is uncertain, since the initial high level will be rapidly reduced as a result of dilution by tissue fluid, diffusion of peptide away from the injection site, and enzymatic degradation. Thus there may be no peptide present for much of each 24-h period, and the average peptide concentration may well be in the physiological range. It is quite clear, however, that the concentration of adrenomedullin required to produce a half-maximal effect on osteoblast proliferation in vitro is comparable to the circulating concentrations of this hormone found in vivo. This, together with the present studies in adult mice, suggests that adrenomedullin is likely to be a physiological influence on osteoblast proliferation in vivo.

Although the physiological role of this hormone is still being defined, the present data suggest that it might extend beyond vasodilation and salt and water metabolism to include regulation of bone metabolism. This is supported by recently published evidence for the expression of adrenomedullin and its receptor in high concentrations in osteoblasts during the later stages of
Adrenomedullin increases bone mass

**Fig. 6.** A: dose-response relationship for [3H]thymidine incorporation into neonatal mouse calvaria after 48 h of treatment with ADM. Data are means ± SE. There is a significant treatment effect (P = 0.0002). *Significantly different from control. B: percentage of 45Ca-release/bone from neonatal mouse calvaria after 48 h of treatment with ADM. Data are means ± SE. There was no significant change from control.

Adrenomedullin exists with respect to their effects on bone resorption. Amylin is consistently found to inhibit basal and stimulated resorption in organ culture (2, 28, 33) and to reduce the activity of isolated osteoclasts (1). We have confirmed this action of amylin in both the neonatal mouse calvarial model (30) and in vivo in the adult mouse (3) but could detect no suppression of resorption by adrenomedullin in these models. Consistent with this is the failure of adrenomedullin to produce hypocalcemia in the rat (35). In fact, the present in vivo studies show the opposite trend, probably because of the coupling of resorption to formation, which comes into play over the longer time course of this experiment. The dissociation of the osteoblast and osteoclast effects of amylin and adrenomedullin suggests that these actions are mediated by different receptors. One possibility would be that amylin produces its inhibition of bone resorption via the osteoclast’s calcitonin receptor, whereas the anabolic effects on bone of both amylin and adrenomedullin are mediated by a distinct receptor on the osteoblast. These possibilities will need to be tested by binding studies.

The present data include a detailed study of the structure-activity relationships of adrenomedullin. Previously, such data have only been available for its effects on vascular smooth muscle. In that tissue, removal of up to 15 NH2-terminal amino acids has little effect on the peptide’s vasodilator activity (9, 11, 32). However, loss of the ring structure either by more extensive NH2-terminal truncation or by formation of a linear analog not containing the disulfide bond eliminates specific binding and cAMP formation in rat vascular smooth muscle cells (9, 32). Removal of the COOH-terminal amide also results in loss of agonist activity (9), and NH2-terminal peptides are inactive (9, 11). In contrast, activity in osteoblasts is preserved in peptides as short as adrenomedullin-(27—52), suggesting that the disulfide bond and the ring structure created by it are not necessary. This suggests that a different receptor mediates the effects of adrenomedullin in osteoblasts from that in vascular smooth muscle. This dissociation of the vascular and osteotropic effects of adrenomedullin may be very important if its proliferative effects on bone were to be used therapeutically. The diminution in activity of the adrenomedullin molecule after reduction of the disulfide bond is not surprising, since this will change the conformation of the peptide and interfere with its receptor binding even if the sequence with affinity for the receptor is intact.

The activity of these short adrenomedullin fragments is also surprising when comparison is made with amylin. When the ring is removed from amylin, the resulting peptide [amylin-(8—37)] has no agonist activity on osteoblasts and is in fact an antagonist. This suggests that activation of the osteoblast amylin recep-
Adrenomedullin is dependent, in part, on the COOH-terminal sequence of the peptide and that modification in this region can restore activity lost as a result of NH2-terminal truncation of the molecule. This observation may be relevant to the design of amylin analogs for use in bone and other tissues.

In conclusion, the present studies demonstrate that adrenomedullin is a potent stimulator of osteoblast proliferation and protein synthesis that increases bone mass and is active at periphysiological concentrations. This raises the possibility that it may have a role in the regulation of bone metabolism both in health and disease. One of the major challenges in osteoporosis research at present is the development of a practical therapy that increases bone mass by stimulating osteoblast activity. The further definition of the bone actions of the amylin-adrenomedullin class of peptides is a promising new research direction in this important area. The present data suggest that the development of a relatively short peptide from this family, which has a selective anabolic action on bone, is a real possibility.

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