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A quantitative description of components of in vitro morphometric change in the rat osteoclast model: relationships with cellular function

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Abstract. We describe the in vitro morphometric changes shown by rat osteoclasts that accompany their functional responses to the application of a range of regulatory agents of known physiological importance. We introduce a cellular motility parameter, μ, which was defined through a quantification of retraction-protrusion behaviour. This was used in conjunction with a net cell retraction, ϱ, which is derived from the change in total cell area following the application of an agent. These terms were used together for the description of cellular motility changes in response to specific cellular regulatory agents. The definition of retraction-protrusion was normalised against control cell area, to give a dimensionless variable independent of the net cell retraction. Thus, mutual terms present in either descriptor cancelled when the complementary parameter was held constant. Furthermore, the descriptor, μ remained time-invariant for extended intervals (around 20 min) even when ϱ was varying following cell introduction into culture. Interventions also with substances known to modify osteoclast function, were capable of altering each descriptor, to different extents. Thus elevation of the extracellular Ca^{2+} concentration ($[Ca^{2+}]_e$) at the osteoclast calcium "receptor" altered ϱ without changes in μ. In contrast, the polypeptide amylin (250 nM), within 20 minutes of application, elicited a marked change in μ, but only a relatively small change in ϱ. Finally, human calcitonin treatment (300 pM) influenced both descriptors. When combined together, these morphometric findings accordingly offer complementary descriptions of visible cellular changes in response to added agents of physiological relevance. Such an approach may be useful in the analysis of structure-function relationships in osteoclasts or other cell systems particularly in correlations between quantitative structure and functional responsiveness.

Key words: Cell shape – Cell motility – Osteoclasts – Bone resorption

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Introduction

The osteoclast is unique in its ability to resorb bone. The recent development of methods for isolation and culture of osteoclasts has allowed detailed studies of the mechanisms of bone resorption and its control. Much interest has been directed to the roles of hormones, cytokines and ions in regulation of osteoclast function. For example, progress has been made in understanding the mode of action of calcitonin, the main circulating regulator of osteoclast function (review: Chambers 1991). It has also been established that stimulators of osteoclastic bone resorption, including parathyroid hormone and cytokines, do not directly modulate osteoclast activity, but do so via the osteoblast. More recently, it has been shown that the extracellular concentration of calcium ions, generated locally as a result of osteoclast activity, can directly regulate osteoclast function, providing a possible feedback control mechanism (Zaidi et al. 1989).

The resorptive activity of osteoclasts is dependent upon the ability of these cells to secrete hydrolytic enzymes into the sealed zone or "hemivacuole" formed between the ruffled margin of the bone resorbing cell and the bone surface. Thus, osteoclastic resorption may depend, at least in part, upon the various types of motions exhibited by the cell itself and the plasma membrane. Changes in cell morphology induced by physiological and pharmacological agents might therefore interfere with the formation and maintenance of the resorptive hemivacuole (review: Zaidi 1990a).

In view of the close relationship between functional and morphological changes, and alterations in both in response to applied external agents, it seemed useful to develop a quantitative analysis of cell structure. The system where simple and rigorous description of changes in cell shape can be described in terms of simple length (or tension) along a single geometric axis, is skeletal muscle. However, quantitative descriptions of cell shape along at least two, if not three dimensions, is considerably more complex. There is an extensive literature on analysis of cell shape (see e.g. Alt and Hoffman 1991; Noble and

Levine 1986). This paper attempts a representation of in vitro changes in osteoclast behaviour by introducing ad hoc descriptions of cell morphology whose values correlate with changes in functional activity. Thus we introduce a combined description that employs two parameters, an "area descriptor" (ϱ) and a "motility descriptor" (μ), for osteoclast morphology. This approach is considered in context with the previous quantitative descriptions of biological structure in the Discussion. We explored the extent to which simultaneous use of two parameters of cell morphology is more satisfactory than the use of a single variable alone, and related these two parameters to the behavioural changes produced by agents with known effects on cell function.

Materials and methods

Materials

Human amylin and human calcitonin, (kindly provided by Drs. T. J. Rink, Amylin Corporation, San Diego, U.S.A., and W. Rittel, Ciba-Geigy, Basel, Switzerland, respectively), were dissolved in distilled deionized water containing acetic acid (0.01%, v/v; "Aristar" grade; BDH, Dorset, U.K.) and bovine serum albumin (1%, w/v; BSA; Sigma, Dorset, U.K.; "recrystallized"), then lyophilized and stored at −70 °C. These were reconstituted in phosphate buffer (0.05 mM; pH 7.4) containing BSA (0.01%, w/v) and aprotinin proteinase inhibitor (200 000 Kallikrein Inhibitor Units or KIU l^{-1}, "Trasylol"; Bayer, Newbury, Berks., U.K.). All chemicals were also of the highest available grade, and were purchased from either Sigma, U.K. or British Drug Houses, U.K. Tissue culture media and heat-inactivated fetal calf serum (FCS) were purchased from Flow Laboratories, UK, Ltd. (Uxbridge, Middlesex, U.K.). Osteoclasts were isolated in Medium 199 buffered with N-2-hydroxyethyl piperazine-N′-2-ethane sulphonic acid (HEPES) and supplemented with FCS (10% v/v), benzyl penicillin (Glaxo, Middlesex, U.K.; 100 IU l^{-1}) and streptomycin (Glaxo; 100 mg l^{-1}) (referred to as Medium 199).

Osteoclast isolation and culture

Newborn Wistar rats were killed by cervical dislocation (Schedule 1 procedure of the U.K. Home Office) and their femora and tibiae obtained. The adherent soft tissues were removed and the bones cut across their epiphyses in Medium 199. Osteoclasts were mechanically disaggregated by curetting the bones of each rat into 1 ml Medium 199 and agitating the suspension with a pipette. The osteoclast suspension was dropped onto plastic petri dishes (35 mm; Sterilin, Middlesex, U.K.) and the cells were allowed to sediment and attach at 37 °C for 20 minutes in 10% humidified CO_2 (Fig. 1).

Instrumentation

The equipment used was built upon an inverted phase contrast microscope (Diaphot, Nikon U.K. Ltd., Telford, Shrops., U.K.) with phase contrast and Nomarski optics and a humidified incubation chamber. The signal from the sideport of the microscope passes via a charge coupled device (CCD) camera (Model WECD10, National Panasonic, London, U.K.) to a 480-hour mode time-lapse video recorder (Mitsubishi, Tokyo, Japan). The taped video records could be played back for subsequent analysis at 120 times the recording speed. The composite signal from the video recorder also feeds into a 256-grey level imaging system (Sight Systems, Newbury, Berks., U.K.), via a British National connector, centered on a 60 MByte hard disc IBM microcomputer (IBM, Harrow, U.K.) containing a video interface card.

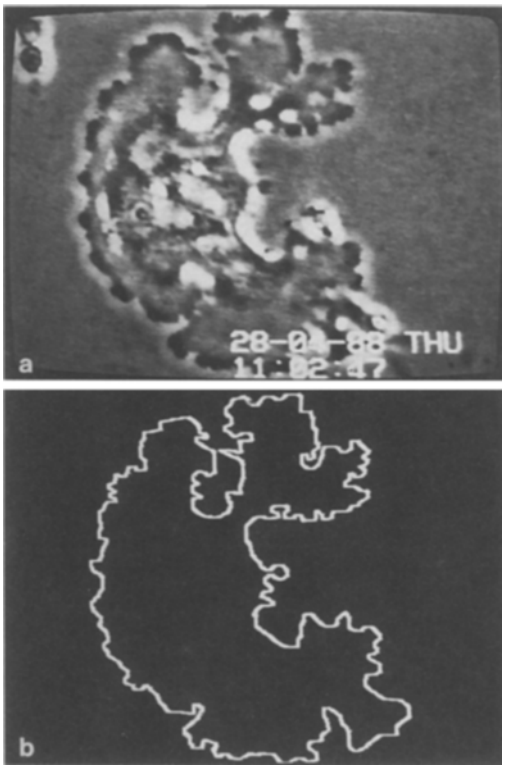

Fig. 1. A digitised grey image of an osteoclast on plastic substrate, 30 minutes following its isolation from bone **a**. Ruffling at the cell margin is evident. The image has been redigitised using a binary overlay **b**. Phase contrast × 40

Image processing

Osteoclast images were digitised at fixed time intervals using a standard software package. The resulting grey images were stored in the hard disc of the microcomputer as sequential files. A graphics program was then used to generate outlines for each grey image. During this procedure, each grey image was retrieved in sequence. The position of each image was standardized by entering a pair of reference etch marks in the examined field using a mouse (Microsoft, New York, U.S.A). The mouse was then used to draw around the cell margin allowing the generation of a 2-pixel wide binary outline. Figure 1 illustrates one such captured image, together with the corresponding 2-pixel binary outline. This outline was record-

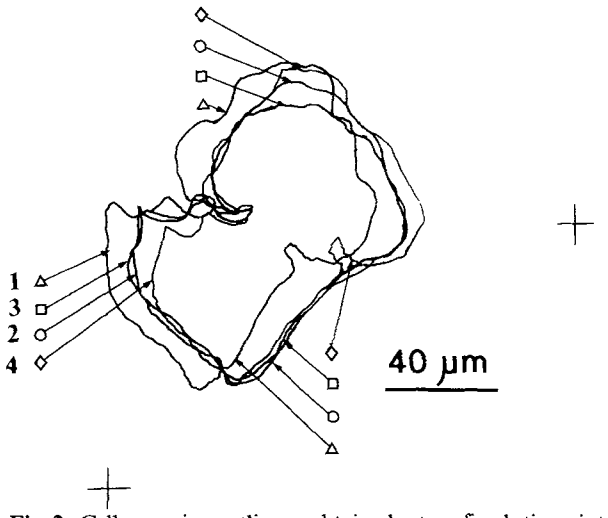

Fig. 2. Cell margin outlines, obtained at a fixed time interval, $\Delta t = 2$ min, have been retrieved in sequence and overlaid upon the previous image to illustrate the method for surface area measurements and retraction-protrusion activity at the cell margin

ed as two files, containing respectively the binary image data and information in the form of either ASCII characters (number of reference points for each image, x, y-coordinates of each reference point and start of outline and number of points on outline) or the outline itself, stored as a vector list in form of binary data. Information contained within the latter file could be decoded for display to ensure that a pair of reference etch marks previously made in the examined field, were superimposed as successive digital images. Results of such a tracing procedure taken together with reference marks is shown in Fig. 2.

Empirical morphometric analysis

An empirical morphometric analysis was performed through the comparison of successive traces of cell outlines obtained at the image capture intervals. First, cell spread area, $A(t)$ at each time t was obtained. It was expressed as a percentage of mean control area $\langle A(t_0)\rangle$ (mean $\pm$S.E. of the mean) obtained over a 20 min interval prior to stimulus application. This yielded a measure of the overall degree of cell area change. Second, a tracing of each cell obtained at time t bounding the set of pixels was overlaid on the previous outline at time, $(t-\Delta t)$. The degree of cell retraction, $[\Delta r]$, was determined from the set of pixels enclosed within the area $[A(t-\Delta t)]$, but excluded from $[A(t)]$; conversely, cell protrusion, $[\Delta p]$, was determined by the set of pixels falling within $[A(t)]$ but not $[A(t-\Delta t)]$.

Validation

The precision of motility and area measurements depend upon the accuracy of the image processing and analysis system. This was tested by determining the precision of measurements in a variety of situations. Briefly, the numerical aperture of the microscope objective (numerical apertures 0.55 versus 0.85), type of camera (colour CCD, 10 lux, signal: noise ratio -46 dB versus monochrome CCD, 3 lux, signal to noise ratio -46 dB), video recorder or monitor did not significantly influence the precision of area or motility measurements. In each case, the coefficient of variation was $<0.2\%$. The repeated acquisition, as a digitised grey image, of the same video image, followed by redigitisation of the cell margin to create a data file produced a variation coefficient of 0.43% for area measurements, and 2.30% for motility measurements. When the cell margin of any one grey image was repeatedly redigitised to create a file, this gave a coefficient of variation of 0.32% for area measurements and 4.68% in motility measurements ($n=10$ digitisations). These errors were insignificant relative to the magnitude of changes in the measured parameters observed in response to added agent. These responses were analysed by one way Analysis of Variance, and differences were considered significant where $p<0.01$.

Results

The quantitative description of dynamic morphological changes

There are relatively few available means for the a priori quantitative analysis of dynamic changes in cell form as a function of time (e.g. Dunn and Brown 1986). The present treatment was developed from a set of fundamental descriptive variables, viz, cell area, $A(t)$, cell retraction, $\Delta r(t)$ and cell protrusion, $\Delta p(t)$. These are independent of cell position and have common dimensions of (length)^2. $A(t)$ was determined from the set of image pixels covered by a given cell at time, t. Cell protrusion was determined from the pixel set covered by the cell at time element, t, but not previously covered at time, $t-\Delta t$. Cell retraction, written as a negative variable, was conversely, determined from that pixel set, previously covered at time element, $t-\Delta t$, but not at time, t. Terms of retraction or protrusion, or net changes in cell area in unit time, Δt as fractions of pre-existing cell area had dimensions identical to those of first-order rate constants. Thus:

$$\text{Instantaneous protrusion} = \frac{1}{\Delta t} \cdot \frac{\Delta p(t)}{A(t-\Delta t)}$$

$$\text{Instantaneous retraction} = \frac{1}{\Delta t} \cdot \frac{\Delta r(t)}{A(t-\Delta t)}$$

Instantaneous rate of change of total area

$$\frac{1}{\Delta t} \cdot \frac{\Delta A(t)}{A(t-\Delta t)}$$

The absolute values for the first two quantities $[\Delta p(t)]$ and $[\Delta r(t)]$ above were combined to yield an overall normalized "motility descriptor":

$$\mu(t) = \frac{[\Delta p(t)] + [\Delta r(t)]}{A(t)}$$

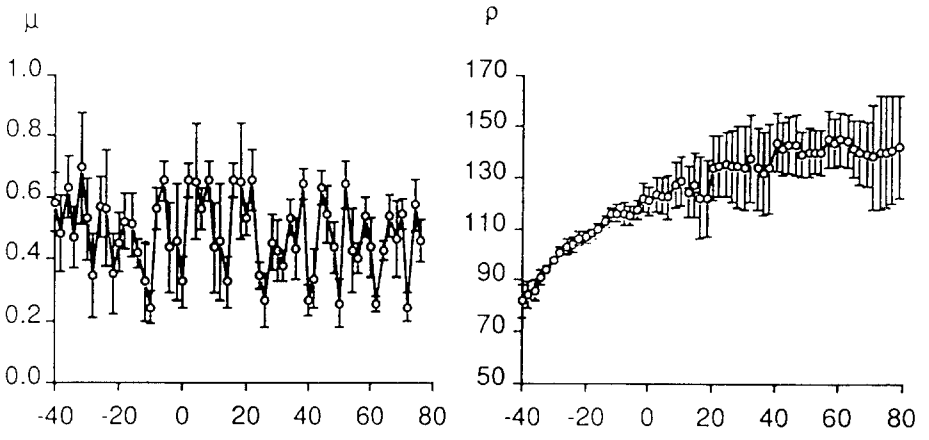

Fig. 3. The dependence of the motility and area "descriptors", μ and ϱ, following introduction of ostoclasts into culture. μ represents cell motility, $([\Delta p]+[\Delta \varrho])/A(t)$, whilst ϱ represents cell spread area, normalized to control cell spread area or $A(t)/\langle A(t_0)\rangle$. Results are expressed as mean ± standard error of the mean, $n=10$ cells

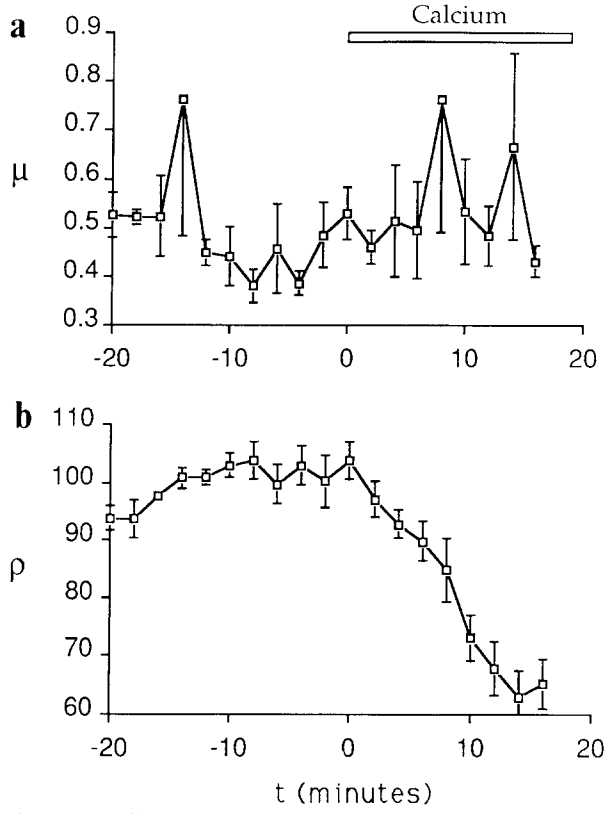

Fig. 4. The effect upon the motility (**a**) and area (**b**) "descriptors", μ and ϱ, of an application of 20 mM-[extracellular ionised calcium] (Calcium), at time element, $t=0$ minutes. μ represents cell motility, $([\Delta p]+[\Delta r])/A(t)$, whilst ϱ represents cell spread area, normalized as a percentage of control cell spread area or $A(t)/\langle A(t_0)\rangle$. Results are expressed as mean ± standard error of the mean, $n=10$ cells. Control cell spread area, $A(t_0)$, was estimated from 10 recordings obtained prior to exposing the cells to the agent

Conversely, in the absence of changes in motility $\mu(t)$, we defined an "area descriptor":

$$\varrho(t)=\frac{A(t)}{\langle A(t_0)\rangle}$$

This was also dimensionless; it was normalized to a set of control readings taken preceding the addition of external agents.

It is important to note that $\varrho(t)$ and $\mu(t)$ are independent measures respectively, of net reduction of overall cell spread area and of retraction-protrusion activity at the cell margin. Thus, the "area descriptor" ϱ, is a consequence of the net *difference* between cell margin retraction and protrusion, Δp and Δr. In contrast, the 'motility descriptor, μ, is derived from the *sum* of the positive magnitudes of retraction and protrusion, Δp and Δr. Furthermore ϱ is normalised to $A(t_0)$, the cell spread area prior to any applied perturbation. In contrast, μ is normalised to the cell area, $A(t)$ at instant, t.

$\mu(t)$ and $\varrho(t)$ achieve stationary values over different time courses following introduction into culture

A necessary justification for a description involving use of both parameters defined above rather than only one parameter would require the demonstration of alterations in one in preference to the other descriptor under particular functional conditions. Figure 3 illustrates the results of runs of determinations of $\mu(t)$ and $\varrho(t)$ following introduction into culture.

Figure 3 illustrates that $\varrho(t)$ reached a stationary value over 40 min following introduction into culture. At such earlier times, values of $\varrho(t)$ were small but this was followed by an increase in spread. Note that values of $\varrho(t)$ in Fig. 3 were normalized to the mean initial area $A(0)$ obtained over the first 20 min following introduction. In contrast, $\mu(t)$ appeared to maintain similar values throughout the experimental period. Thus, variation in one parameter was possible in the face of constant values in the other.

Calcium "receptor" activation alters $\varrho(t)$ but not $\mu(t)$

Earlier papers have demonstrated that an elevation of the extracellular calcium concentration, $[Ca^{2+}]_e$, activates a putative calcium "receptor" on the osteoclast leading to functional inhibition (Zaidi et al. 1989; Zaidi 1990b). We have examined the effect, on retraction and motility of isolated osteoclasts, of an application of 20 mM-$[Ca^{2+}]_e$ at time, $t=0$ min ($n=10$ cells). Figure 4a shows the values of the normalized motility, $\mu=([\Delta p]+[\Delta r])/A(t)$ with

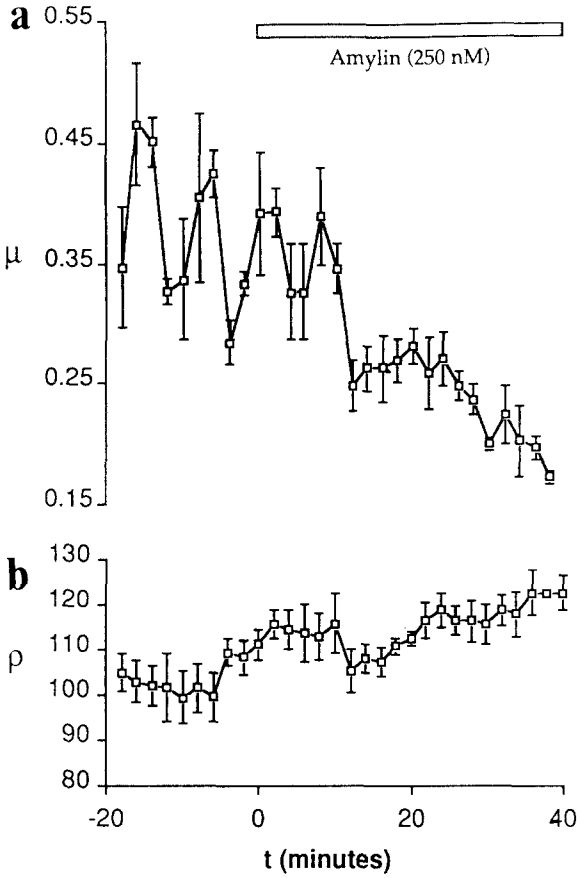

Fig. 5. The effect upon the motility (**a**) and area (**b**) "descriptors", μ and ϱ, of an application of 250 nM-[amylin], at time element, $t=0$ minutes. μ represents cell motility, $([\Delta p]+[\Delta r])/A(t)$, whilst ϱ represents cell spread area, normalized as a percentage of control cell spread area or $A(t)/\langle A(t_0)\rangle$. Results are expressed as mean $\pm$ standard error of the mean, $n=10$ cells. Control cell spread area, $A(t_0)$, was estimated from 10 recordings obtained prior to exposing the cells to the agent

time. The records indicate an absence in this parameter, of a significant rate of change from the steady values preceding the application of test solution. This indicates an absence of change in activity at the cell margin in response to elevated $[Ca^{2+}]_e$. Thus, the parameter, μ, offers a useful measure, as a single number, of the observed activity at the cell margin at a given time, t.

In contrast, Fig. 4 b shows a dramatic reduction in the value of the "area descriptor", $\varrho=A(t)/\langle A(t_0)\rangle$, by 40% of its pretreatment control value (half-time of the response $=8.9$ min). A change in ϱ in response to elevated $[Ca^{2+}]_e$ has previously been shown to be associated with an elevation of $[Ca^{2+}]_i$ and with a reduction in osteoclast function. Furthermore, the results here, indicate that a marked reduction in ϱ is compatible with relatively little corresponding change in μ. This emphasizes the importance of using both descriptors in the analysis of osteoclast shape.

Amylin preferentially alters $\mu(t)$ rather than $\varrho(t)$

We reported recently that exposure of isolated osteoclasts to the 37 amino acid peptide, amylin, leads to the inhibition of osteoclastic bone resorption (Alam et al. 1991). In this study, we examined the effect, on both motility and retraction of isolated osteoclasts, of an application of 250 nM-[amylin] at time, $t=0$ min ($n=10$ cells). Figure 5 a follows the normalized motility, $\mu=([\Delta p]+[\Delta r])/A(t)$ with time. Before amylin treatment, values for μ remained relatively constant, but following treatment declined towards the abscissa. Logistic regression analysis revealed a significant ($p<0.001$) first-order regression of response over time (correlation coefficient, $r=0.89$, slope $=-3.39$; half-time of response $=19$ min). The control (pretreatment) data did not regrees on time ($p=0.9$) and a poor correlation was found, between the two parameters ($\varrho<0.1$; Analysis of Variance). These results are in contrast to those shown in Fig. 4 a indicating an absence of change in activity at the cell margin in response to elevated $[Ca^{2+}]_e$.

Analysis of the "area descriptor", $\varrho(t)$, expressed as a percentage of $\langle A(t_0)\rangle$, in response to a 40 minute exposure to 250 nM-[amylin] revealed no change with respect to pretreatment recordings (Fig. 5 b). When analysed by logistic regression analysis, the data did not regress over time ($p>0.1$), and response regression lines had positive slopes with values exceeding unity.

Calcitonin affects both descriptors $\mu(t)$ and $\varrho(t)$

It is known that exposure of isolated osteoclasts to the 32 amino acid hormone, calcitonin, leads to a strong inhibition of osteoclastic bone resorption. Thus, we have examined the effect, on the shape of isolated osteoclasts, of the application of 300 pM-[calcitonin] at time $t=0$ min ($n=7$ cells). Figure 6 a follows the normalized motility, μ with time. Before calcitonin treatment, values for μ remained relatively constant, but following treatment, they declined sharply to the abscissa over a half time of approximately 15 min. Logistic regression analysis revealed a significant ($p<0.001$) first order regression of response over time (correlation coefficient, $r=0.9$; slope $=-3.0$).

In contrast, Fig. 6 b shows a reduction in the value of the "area descriptor" $\varrho=A(t)/\langle A(t_0)\rangle$, to 60% of its pretreatment control value over a half time of about 26 min. Logistic regression analysis revealed a significant ($p<0.001$) first order regression of response over time (correlation coefficient, $r=0.96$). It is notable that changes in ϱ and μ, observed in response to calcitonin, follow different timecourses. The effects of calcitonin on both descriptors may also be compatible with the high osteoclast-inhibitory potency of the peptide, and hence its physiological role in osteoclast control.

Discussion

This paper introduces a quantitative description of dynamic motile processes of the rat osteoclast in culture. We have achieved an analysis of retraction and protrusion of the cell margin in terms of a set of dimensionless variables, thus extending our earlier study where initial attempts were made to characterize morphometric changes

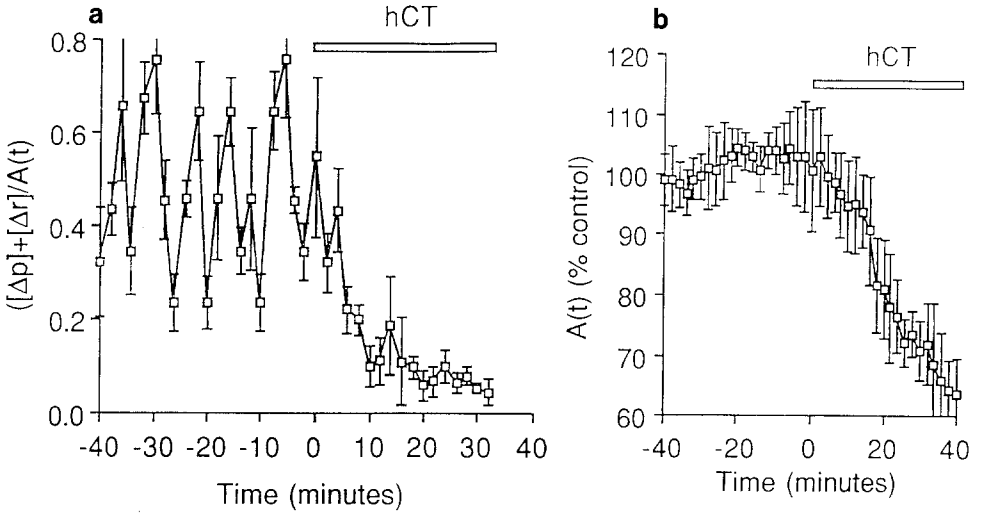

Fig. 6. The effect upon the motility **a** and area **b** "descriptors", μ and ϱ, of an application of 300 pM-[human calcitonin] at time, $t=0$ min. μ represents cell motility $([\Delta p]+[\Delta r])/A(t)$, whilst ϱ represents cell spread area normalized as a percentage of control cell spread area or $A(t)/\langle A(t_0)\rangle$. Results are expressed as mean ± standard error of the mean ($n=7$ cells). Control cell spread area $A(t_0)$ was estimated from 10 recordings obtained prior to exposing the cells to the agent

in response to the application of pharmacological reagents (Zaidi et al. 1990). This has resulted in a more complete and quantitative assessment of the responsiveness of isolated osteoclasts, on this occasion, to agents of known physiological relevance.

Quantitative descriptions of biological structure have been made on earlier occasions. These have included estimations of numbers of repeated structures, such as cells, in a defined microscopic field, and derivations of volume or area parameters through point grid sampling methods to yield results averaged over the population examined (review: Aherne and Dunnill 1982; Williams 1977). In addition, there is an extensive literature concerning parameters that describe individual cells. These include harmonic and decomposition analyses of cell shape (see e.g. Alt and Hoffman 1991), which can be applied to biological data through computational methods (Noble and Levine 1986).

Dunn and Brown (1986) have also reported quantitative studies examining the static shape of chick heart fibroblasts cultured on substrata containing geometrically rule grooves. They employed moment-generating parameters to analyse digitally imaged shapes of fixed cells, an approach that has developed from the fields of image analysis and computer pattern recognition. Their study demonstrated progressive changes in cell shape and orientation over a range of groove parameters, indicating that these changes were attributable entirely to a simple geometric transformation of cell shape. Such an approach was extended to studies of dynamic shape changes in fibroblasts in vitro following addition of tumour-producing phorbol esters (Brown et al. 1989).

The present study also considers dynamic as opposed to static cell behaviour. As in the formal analyses of Dunn and Brown (1986), we have chosen descriptors that are independent of cell position and orientation with respect to a Cartesian coordinate system. We have introduced a description of motility that is primarily concerned with the quantification of dynamic changes at the cell margin in addition to representation of changes in bulk area properties through the extent of cell retraction. Thus, our investigations involve the simultaneous observation of two descriptors that characterize retraction-protrusion of the margin ("motility descriptor", μ) as well as net changes in cell spread area ("area descriptor", ϱ). These descriptors are separable in biophysical terms through the simple definitions introduced here. Thus, a motility change, characterised by a gradual cessation of retraction-protrusion behaviour, could take place in the absence of a change in area. Conversely, a net retraction response leading to a change in cell spread area need not be accompanied by a change in total cell motility, which in this case, is the numerical sum at a given instant, of the magnitudes of the retraction and protrusion behaviour observed at the cell margin. This latter separation of properties has been reinforced by the experimental demonstration of the selective effects of $[Ca^{2+}]_e$ elevation, and amylin and calcitonin on the two descriptors, respectively. Thus, we consider our approach will be useful, at the very least, in the analysis of morphometric responses of the osteoclast system.

We believe that such descriptions of osteoclast shape may have important physiological correlates for the bone resorptive process and its control. For example, it is thought that motility provides the potential for bone resorption by osteoclasts, and that diminished bone resorption can result directly from just the inhibition of osteoclast motility. This functional correlate may be related to changes in the velocity of movement of cytosolic granules containing resorptive enzymes (Zaidi 1990a). Likewise, net cell retraction could reduce bone resorption by disrupting the sealed zone of close adhesion between the osteoclast ruffled border and the bone matrix. The creation and maintenance of such a sealed compartment of resorptive "hemivacuole" is essential for the resorptive episode to occur. Thus, an intervention on either motility or spread area may constitute an effective intervention on bone resorption (Zaidi 1990a). This knowledge may lead to important therapeutic consequences for diseases characterised by increased osteoclastic activity.

We have also used this quantitative approach to study the effects of a range of agents having known physiologically relevant actions on the osteoclast. In our earlier morphometric study, we adopted empirical descriptions

of cell behaviour in terms of a quiescence (Q effect) and a retraction (R effect) response. These distinct response patterns had suggested the existence of parallel intracellular signalling pathways in the osteoclast. Here, we report that the area or motility descriptors, ϱ and μ, can vary to different extents, particularly following addition of modulatory agents. Thus activation of the putative osteoclast calcium "receptor" by an elevation of $[Ca^{2+}]_e$ selectively altered the "area descriptor", ϱ. It is notable however, that despite having no demonstrable influence on cell motility, μ, an increased $[Ca^{2+}]_e$ was found to be strongly inhibitory to osteoclastic bone resorption. It is thought that the effect of calcium is a physiological response, providing a possible feedback control mechanism. It is also notable that the calcium ionophore, ionomycin, and a number of substances that elevate osteoclast $[Ca^{2+}]_i$, including verapamil and perchlorate, are known to cause net cell retraction (review: Zaidi 1990b). This may suggest a functional relationship between the "area descriptor", ϱ, and $[Ca^{2+}]_i$. In contrast, amylin preferentially reduced motility, μ, without an influence on cell spread area, ϱ. This might be compared with earlier findings with cholera toxin and dibutyryl cyclic AMP.

In conclusion, we have introduced a hitherto novel approach for the description of cell shape using mathematically independent and experimentally separable parameters. This extends our earlier empirical studies (Zaidi et al. 1990), and those relating to static (Dunn and Brown 1986) and dynamic (Brown et al. 1989) cell properties. The resulting formalisms have been applied successfully to the rat osteoclast model to define the functional responses of osteoclasts to a variety of physiological agents. In general, such quantitative descriptions of cellular dynamic processes may well offer a useful approach for the study of structure-function relationships in a range of motile eukaryotic cells.

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