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Effect of vasoactive peptides in *Tetrahymena*: chemotactic activities of adrenomedullin, proadrenomedullin N-terminal 20 peptide (PAMP) and calcitonin gene-related peptide (CGRP)

László Kőhidai[1] · Katalin Tóth[1] · Paul Samotik[1] · Kiran Ranganathan[1] · Orsolya Láng[1] · Miklós Tóth[2] · Heikki Ruskoaho[3]

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Abstract Adrenomedullin (AMD), proadrenomedullin N-terminal 20 peptide (PAMP) and calcitonin gene-related peptide (CGRP) were studied for chemotaxis, chemotactic selection and G-actin/F-actin transition in *Tetrahymena*. The aim of the experiments was to study the effects of two different peptides encoded by the same gene compared to a peptide related to one of the two, but encoded by a different gene, at a low level of phylogeny. The positive, chemotactic effect of ADM and the strong negative, chemorepellent effect of PAMP suggest that in *Tetrahymena*, the two peptides elicit their chemotactic effects via different signalling mechanisms. The complexity of swimming behaviour modulated by the three peptides underlines that chemotaxis, chemokinesis and some characteristics of migratory behaviour (velocity, tortuosity) are working as a sub-population level complex functional unit. Chemotactic responsiveness to ADM and CGRP is short-term, in contrast to PAMP, which as a chemorepellent ligand, has the ability to select sub-populations with negative chemotactic responsiveness. The different effects of ADM and PAMP on the polymerization of actin networks show that the microtubular structure of cilia is more essential to chemotactic response than are transitions of the actin network. The results draw attention to the characteristic effects of vasoactive peptides at this low level of phylogeny.

Keywords Adrenomedullin · CGRP · Migration · PAMP · Tetrahymena

Introduction

Unicellular eukaryotes contain molecules similar to vertebrate hormones [1, 2], have receptors [3, 4] and signal transduction systems [5] through which they can react to vertebrate hormones. It was believed that food receptors are used (or transformed) for signal recognition processes [6]; this is supported by the fact that dipeptides are also well recognized and distinguished [7]. It is believed that this discriminatory capacity is combined with hormonal imprinting at this very low level of phylogeny [3, 4] and provides the basis for the parallel evolution of hormones and their receptors.

In previous experiments, different variants of the vasoactive peptide family of endothelins were studied [8]; these variants have amino acid sequence homology. In current experiments, our objective was to study peptides that are encoded by the same gene and that act on the same receptor, but have disparate amino acid sequences. For this purpose, adrenomedullin and related peptides were chosen for eliciting physiological responses in *Tetrahymena*.

In the 1990s, two hypotensive peptides—adrenomedullin (ADM) [9] and proadrenomedullin N-terminal 20 peptide (PAMP)—were discovered [10]. DNA sequence analysis demonstrated that the two peptides are encoded by

László Kőhidai and Katalin Tóth have contributed equally to this work.

✉ László Kőhidai
kohlasz2@gmail.com

[1] Department of Genetics, Cell and Immunobiology, Semmelweis University, Nagyvárad tér 4., Budapest 1089, Hungary

[2] Department of Health Sciences and Sport Medicine, University of Health Science, Alkotás street 44., Budapest 1123, Hungary

[3] Division of Pharmacology and Pharmcotherapy, University of Helsinki, P.O.Box 56, 00014 Helsinki, Finland

the same gene. With respect to the biosynthetic process, the first product is the 185 amino acid containing preproadrenomedullin; further cleavage of a signal peptide from the molecule between Thr_{21} and Ala_{22} yields the 165 amino acid long propeptide which contains the 52 or 50 amino acid sequence of human or rat ADM, respectively. Proteolytic cleavage of preproadrenomedullin at basic amino acid sequence Gly_{42}-Lys_{43}-Arg_{44} provides the 20-residue sequence PAMP, which has no sequence homology to ADM [11, 12]. However, the ADM and PAMP peptides were discovered in pheochromocytoma and in the adrenal medulla [9, 10]. These hormone molecules are present in various human tissues such as the heart, kidney and brain [13], and are also detectable in other mammals such as canines, porcines, rats and mice [14]. Their presence and effects on the eukaryotic ciliate, *Tetrahymena*, have not been studied.

ADM is classified as a member of the calcitonin gene-related peptide (CGRP) superfamily as it shares some homology with CGRP; both molecules possess a characteristic 6-membered ring structure on—or close to—the N-terminus [9]. The specific signalling effect of ADM, elevation of cyclic AMP, is mediated through CGRP-related receptors [15]. They are seven-transmembrane-domain, G-protein-linked receptors. The glycosylation of their extracellular N-terminus is, nevertheless, diverse due to the different intracellular processing of receptors by receptor-activity modifying proteins (RAMPs) [16]. Literature supports the theory that there are several ADM receptors [17]. PAMP has a different structure from ADM, as well as from CGRP; its signalling is based on receptors coupled to G-proteins and modulation of N-type Ca^{2+} channels [18]. Comparison of potency between ADM, PAMP and PAMP (5–20)-NH_2 shows that with respect to a vasorelaxation effect, there is a distinct order present with ADM being most dominant; it also shows that amidation of the C-terminal residue of PAMP is essential for receptor binding [13].

Considering their physiological effects, the hormones elicit their vasodilatory effects through different pathways: ADM decreases vascular resistance by directly acting on vascular smooth muscle cells through CGRP-like receptors, or by releasing nitric oxide from vascular endothelial cells [19], while PAMP acts by inhibiting the release of norepinephrine from adrenergic nerve endings [20]. ADM enhances cardiac contractility via cAMP-independent pathways by releasing intracellular Ca^{2+} stores and activating protein kinase C [21].

ADM has stimulatory effect on the production and release of several substances acting as direct (e.g. IL-6, IL-8) or indirect (e.g. TNF-α) modulators of cell migration [22, 23].

ADM inhibits foetal calf serum, PDGF-induced smooth muscle cell migration [24] and angiotensin II-induced migration through a cAMP-dependent mechanism [25]. Therefore, ADM may potentially play a role as a local anti-migratory factor. By releasing different auto and paracrine vasoactive peptides (ADM, endothelin-1, atrial natriuretic peptide (ANP)), vascular endothelial cells are thought of as potential candidate molecules in the modulation of atherogenesis [24].

The above-mentioned structural characteristics of ADM (and related molecules), as well as their basic physiological effects, make them a suitable tool for studying *Tetrahymena*'s discriminatory capacity with respect to related molecules.

In this study, we examined the above problem with respect to the potential chemotactic effects of ADM, PAMP and CGRP on the eukaryotic ciliate model cell, *Tetrahymena pyriformis* [26]. The model was chosen in order to study the chemotactic potency of these ligands, as chemotaxis is one of the most essential and basic responses of the free-swimming ciliate [27]. Amino acids [28], oligo- [7] and poly-peptides [29] can induce chemotactic responses. The chemotactic potencies of several vertebrate signalling molecules—insulin [30], endothelin-1 [31], ANP [32] and chemokines [33]—exhibit effects at similar concentration ranges in vertebrates and in *Tetrahymena*.

Chemotaxis is only one possibility of the cell in migratory responses in Tetrahymena. In the present study, our objective was to evaluate whether the three vasoactive peptides have joined or independent effect on chemokinetic responses as well as on some markers of the swimming behaviour (e.g. velocity, tortuosity) of the ciliate.

Chemotactic selection is a technique used to investigate the time dependence of inducibility of chemotaxis-related receptors [33]. It applies a chemotactic assay as a probe for selecting cells exhibiting enhanced chemotactic responsiveness. With repeated chemotaxis assays, the technique provides the possibility of evaluating whether the ligand-induced chemotactic response has a short- or long-term character in the organism tested [34]. We, therefore, also used these techniques in our present experiments. Furthermore, considering that the ADM family influences cell migration in mammals, the G-actin/F-actin transition in the cytoskeleton was also studied.

Materials and methods

Cells and culturing

Tetrahymena pyriformis GL cells were cultured in axenic cultures containing 1 % tryptone and 0.1 % yeast extract (Difco, Michigan, USA). Cells were assayed in logarithmic growth phase (48 h). Cell density was 10^4 cells/ml.

Due to the sensitive responsiveness of Tetrahymena to circadian rhythms in swimming, the experiments were carried out in standard illumination and in the same time-period of the days (15:00–18:00).

Hormones and buffers

Peptides tested were human adrenomedullin$_{1\text{-}52}$, PAMP and CGRP (Phoenix Pharmaceuticals Inc.). NaCl-phosphate buffer (PBS), 0.05 M phosphate buffer containing 0.9 % NaCl, at pH 7.2 was used as solvent.

Chemotaxis assay

The chemotactic activity of *Tetrahymena* cells was evaluated using a two-chamber, capillary chemotaxis assay that we modified [35]. In this set-up, an 8-channel-micropipette served as the inner chamber of the system; it was filled with the test substance (buffers containing different concentrations of the peptide to be tested). The outer chamber was a microtitration plate filled with the cells. Incubation time was 20 min—a relatively short time—but necessary in order to measure pure chemotactic response and prevent contamination of samples by chemokinetic responder cells. In the dose–response study, chemotactic response was tested at the concentrations of 10^{-12}–10^{-6} M. Fresh culture medium served as a control substance in simultaneous runs. After incubation, the samples of the inner chamber were fixed using 4 % formaldehyde containing PBS. The number of cells was determined by a Neubauer haemocytometer.

Swimming behaviour: tracking analysis

Swimming behaviour of the cells was video recorded using the Axio-Observer Inverted Microscope (Carl Zeiss MicroImaging GmbH) at a magnification of 2.5×; individual cells were tracked using AxioVision Rel 4.7.1 software. Recorded cells were those having been incubated between 5 and 7 min with either test substance or cell medium (in case of control). 50 µl of sample was pipetted onto each slide and covered with cover slip. Each video recorded cells within the microscope's field of view for 5 s at maximum frame rate (approx. 18 frames per second); data for individual cells were obtained at each frame (one data point per frame). There were on average 25 cells tracked per field of view for each recorded video. Approximately, 3–4 videos were recorded at random positions for each prepared slide; fields of view that contained the slide edge were avoided. The following parameters were determined using recorded position data: (i) mean velocity of the cells (normalized to control) (ii) mean velocity of the fastest and slowest cells and (iii) mean tortuosity of the cell paths (calculated at each frame as the

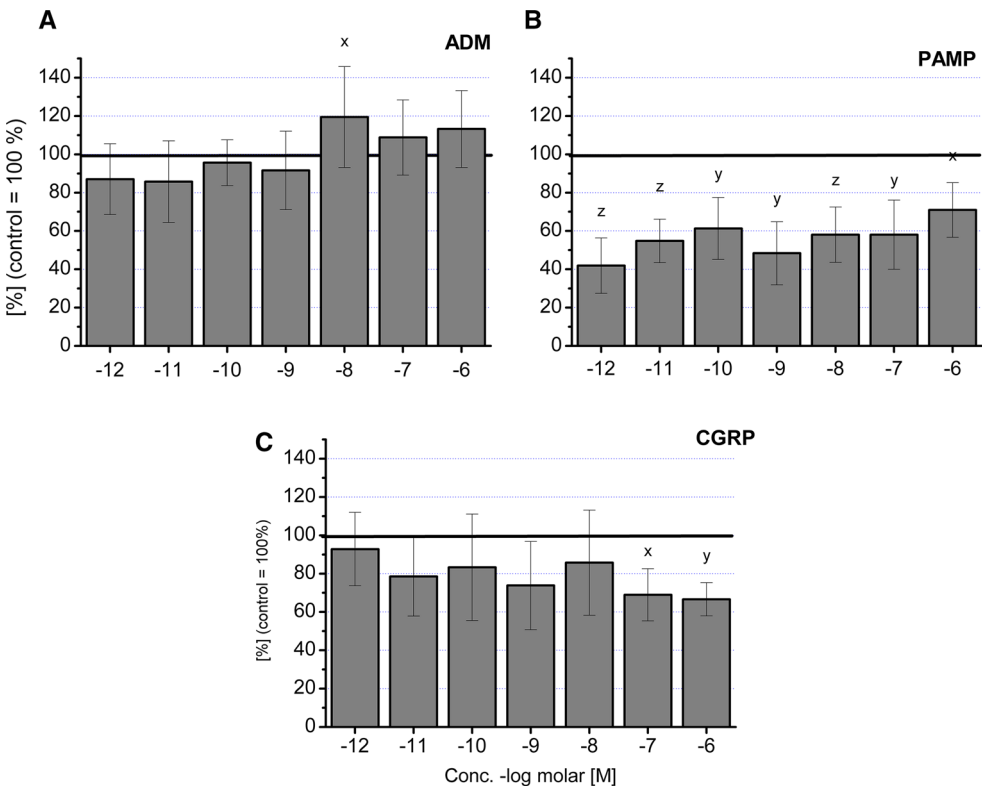

Fig. 1 Chemotactic effects of adrenomedullin (ADM), proadrenomedullin N-terminal 20 peptide (PAMP) and calcitonin gene-related peptide (CGRP) in Tetrahymena—Concentration range study. (*t* test: *x*: $p < 0.05$; *y*: $p < 0.01$; *z*: $p < 0.001$)

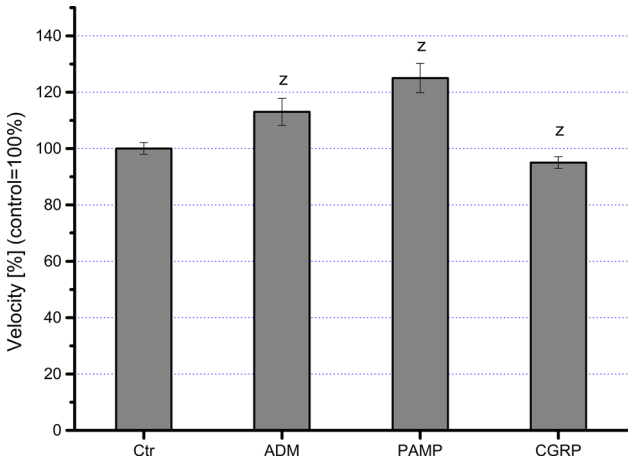

Fig. 2 Computer-assisted swimming analysis of Tetrahymena cells. Effect of adrenomedullin (ADM) (10^{-8} M), proadrenomedullin N-terminal 20 peptide (PAMP) (10^{-8} M) and calcitonin gene-related peptide (CGRP) (10^{-7} M) on the swimming velocity of cells. (*Z* test: *z*: $p < 0.001$)

ratio of the total distance travelled by the cell inclusively between three consecutive data points and the straight distance between the first and third data points).

Chemotactic selection

This technique deals with the chemotactic capacity of different signal molecules to form sub-populations from a mixed culture of cells. First, we applied a chemotaxis assay, as described above. At the end of incubation, positive responder cells were transferred to fresh culture medium for cultivation. Both cultures were selected with known ligand (L), and controls (C) were consecutively transferred in every 48 h. Chemotactic response was determined once again with the following combinations: L/L—cells selected with the given ligand in the first run and assayed to the same ligand in the second run; L/C—cells selected with the ligand in the first run and assayed to the control substance in the second run; C/L—cells selected with the control substance in the first run and assayed to the ligand in the second run; and C/C—cells selected with the control substance in the first run and assayed to the control substance in the second run.

Detection of F-actin content of cells

A fluorescent *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-phallacidin probe (Molecular Probes) (hereafter NBD-phallacidin) was used for the detection of intracellular F-actin in the cells [36]. The cultures were treated with 10^{-12}, 10^{-10} and 10^{-8} M ADM, PAMP and CGRP, respectively. After treatment, the cells were fixed using 4 % formaldehyde containing PBS. The fixed cells were then washed twice with PBS. For permeabilization, a cell membrane treatment of 1 % Triton-X 100 was applied for 5 min, after which samples were washed thoroughly with PBS. To stain F-actin, 6.6 μM NBD-phallacidin was applied for 20 min in darkness. After consecutive rinsing with PBS, the cells were analysed in photomicroscope Nikon Eclipse (binning: none; brightness: 1.1; gamma: 0.63; gain: 2.0; exposition: 10,000 ms; lens: 60×) The fluorescent density was determined using a plate reader (LS50B, Perkin-Elmer; Exc 465 nm, Em 560 nm). In each group of experiments, there were 5 identical samples; 10–10 parallels were measured for each sample.

Statistical evaluation of data

Statistica and Origin 8.0 Pro were used to analyse the data in general. Values of *t*-probe (chemotaxis, chemotactic selection) or *Z*-test (analysis of swimming behaviour) are shown on the figures: *x*: $p < 0.05$; *y*: $p < 0.01$; *z*: $p < 0.001$. Error bars of figures represent ± S.D. values.

Gaussian curves in multi-peak analysis were fitted by using the following formula:

$$y = y_0 + \frac{A}{\frac{w}{\sqrt{\frac{\pi}{2}}}} e^{-\frac{2(x-x_0)^2}{w^2}} \quad (1)$$

Results and discussion

The concentration range study of the three peptides showed that although ADM has a weak chemorepellent or neutral effect at lower concentrations, it acts as a chemoattractant peptide at higher concentrations ($R = 0.88$); 10^{-8}M ADM was able to elicit a maximal chemotactic response in *Tetrahymena* cells (Fig. 1a). PAMP and CGRP had no chemoattractant effect at any of the concentrations tested. Although its chemorepellent character was diverse, PAMP proved to be a strong chemorepellent peptide at all concentrations tested (Fig. 1b); the model cells were rather sensitive to this ligand, as decreasing the concentration of PAMP elicited a more repellent response on the cells ($R = 0.77$). CGRP also acted as a chemorepellent but, in contrast to PAMP, only at higher concentrations (10^{-7}–10^{-6} M); below these concentrations, the chemotactic effect of CGRP was more neutral ($R = -0.90$) (Fig. 1c).

As chemotactic moiety of the three vasoactive peptides proved to be rather diverse, our further objective was to clarify whether the migratory responses are pure vectorial/chemotactic or non-vectorial/chemokinetic responses are also present in a complex migratory response. For this purpose, the computer-assisted tracking analysis was applied which measured effects on swimming velocity as

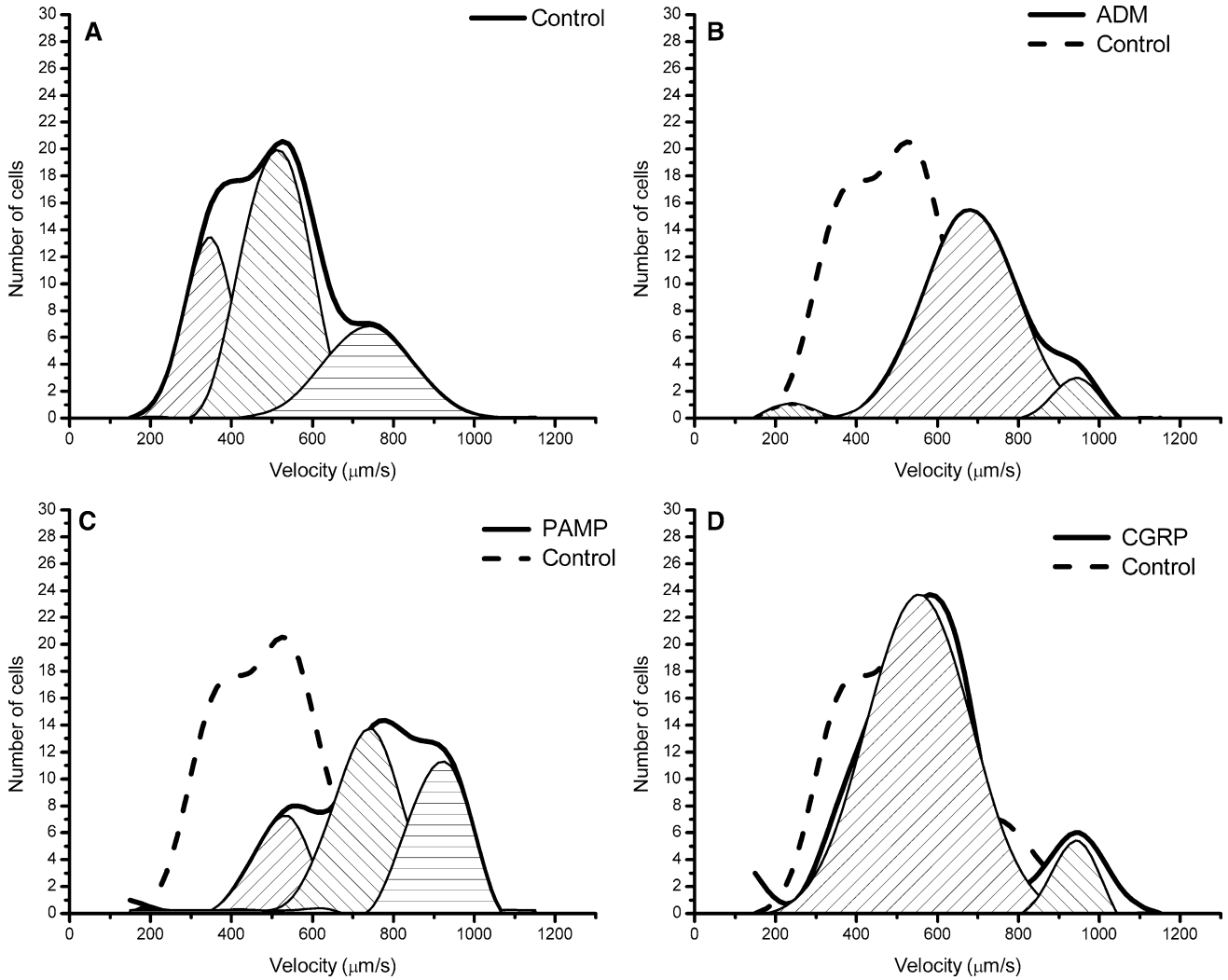

Fig. 3 Effect of vasoactive hormones on swimming velocities of Tetrahymena sub-populations

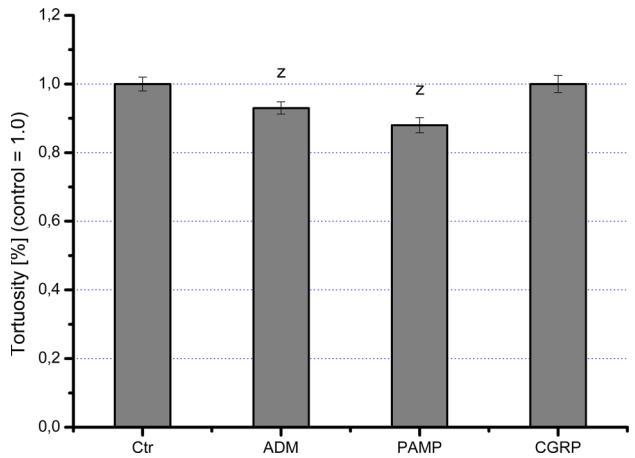

Fig. 4 Computer-assisted tortuosity analysis of Tetrahymena cells treated with adrenomedullin (ADM), proadrenomedullin N-terminal 20 peptide (PAMP) and calcitonin gene-related peptide (CGRP). (*Z* test: *z*: $p < 0.001$)

well as provides a tortuosity analysis of the swimming patterns. In these experiments, the substances were applied in a system where they could elicit effects in a non-concentration gradient dependent manner to the cells.

Our results showed that both adrenomedullin and PAMP could significantly increase the mean velocity of the swimming, while CGRP had a significant but mean velocity decreasing influence on the migration of Tetrahymena (Fig. 2).

As it was presented above, the three hormones proved to have chemoattractant (Adrenomedullin) or chemorepellent (PAMP, CGRP) effects; however, the values of mean velocities refer to that these substances possess chemokinesis inducer (Adrenomedullin, PAMP) or weak but chemokinesis inhibitor (CGRP) effects, too. Nevertheless, these chemokinetic effects were not expressed by the whole populations homogenously (Fig. 3). Even the control population showed inhomogeneity as three sub-

Table 1 Comparison of migratory responses elicited by vasoactive peptides and their sub-population level requirements in Tetrahymena cultures

	Chemotaxis receptor	Effective conc.	Chemo-kinesis	Sub-population	Tortuosity
AMD	Chemoattractant	Single peak	Positive	1 sub-population	$\tau < 1$
PAMP	Chemorepellent	Wide range	Positive	2 sub-populations	$\tau < 1$
CGRP	Chemorepellent	Single peak	Negative	No sub-populations	τ-no change

populations were detectable by Gaussian multi-peak analysis. Size and volume of these sub-populations had a significant difference as a result of short-term treatments with the investigated peptides: one (Adrenomedullin) or two (PAMP) sub-populations possessing higher velocity were distinguishable compared to the control, while CGRP resulted no such significant change on sub-population level.

Computer-based measurement of tortuosity embodies another essential reference of detailed analysis of swimming behaviour in Tetrahymena. Tortuosity itself describes the twisting movement of the cells with a ratio of the distance between the starting point and the point of arrival and the length of the total path measured. Theoretically in a totally linear path, the value of tortuosity is $\tau = 1.0$, while in more tortuous swimming path will result values $\tau < 1.0$. Tortuosity in comparison with the effects of chemotactic or chemokinetic compounds shows τ values are significantly decreased when they are applied in optimal concentrations.

Our present results showed (Fig. 4) that both the chemoattractant ADM and the chemorepellent PAMP—both substances also proved to be positive chemokinetic in this study—resulted significantly decreased tortuosity. In the case of the chemorepellent and negative chemokinetic CGRP, no change in tortuosity was detected.

While the ADM and PAMP effects have a good correlation to the general theories, effect of CGRP remains obscure, while its chemorepellent moiety was significantly weaker than the wide range chemorepellent PAMP. This difference supports our theory that while positive chemokinetic responses—independently of the chemotactic character of the substance—are detectable in distinct sub-populations, negative chemokinetic responses have no sub-population dependence in Tetrahymena cultures. According to the above described theory, it is presumable that significant changes in tortuosity also require sub-population level changes, and sporadic and individual changes remain undetectable for our evaluation systems (Table 1).

The technique of applying an NBD-phallacidin probe, with its high sensitivity to F-actin, was advantageous in detecting the transition of G-actin to F-actin, the process of which is a characteristic marker of migratory processes in amoeboid movement. With respect to effect on cells, relatively higher chemoattractant concentrations of each substance had to be used. *Tetrahymena*-actin has some unique characteristics: It is associated with phalloidin [37], and special actins are associated with the complex microtubular structure of cilia basal bodies [38]. The cilia and its intracellular organizing system (basal body and their submembrane connecting network) constitute a somewhat independent system of locomotion within the free-swimming protozoan. Our present data show that the three peptides tested have a rather negative influence on the activation of the actin network with no special pattern in *Tetrahymena* cells. In the case of ADM and CGRP, concentration-dependent negative effects were detected in relation to the control (Fig. 5a, b). In the highest concentration tested (10^{-8} M), both peptides were strong inhibitors of F-actin transition (ADM 43.47 % ± 1.37; CGRP 61.08 % ± 3.04). The effect of PAMP was also negative, but in contrast to the other two peptides, there was no concentration dependence. These data, however, do not necessarily suggest that cytoskeleton actin transitions and chemotactic effects are parallel processes. The diversity of PAMP's chemorepellent effects contrasted with ADM's effects on the actin network at 10^{-8} M, suggesting that the detected positive or negative chemotactic responses elicited by the three peptides are mediated via "cilia" dominated pathways at this level of phylogeny. In the chemotactic selection study, the responses of selected sub-populations were measured 7 days after selection in the 70th offspring generation from the starter culture. The four groups (C/C, C/L, L/C, L/L) provided data about constant and variable chemotactic responses of sub-populations. For evaluation of these changes, a chemotactic selection coefficient (Ch_{sel}) was used which was calculated from ratios of chemotactic responsiveness of selected and non-selected groups; it considers whether the sub-populations were compared with the control substance or with the medium containing the selector ligand [34]. The coefficient considers each relationship between the four groups studied with one of the ligands.

In the case of ADM and CGRP (Fig. 6a, c), values of Ch_{sel} are close to 1.00 (ADM 1.019 ± 0.035; CGRP 1.137 ± 0.023). Data show that the weak chemoattractant character of ADM detected in the concentration range

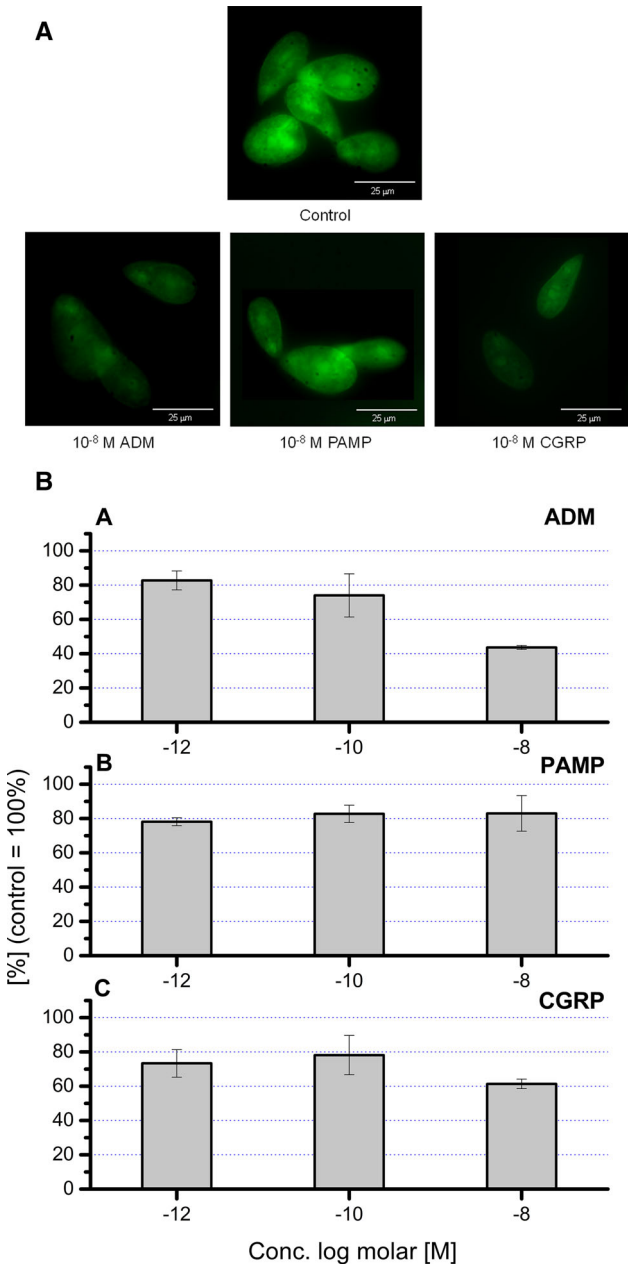

Fig. 5 F-actin content of Tetrahymena detected by NBD-phallacidin. **a** Photographs taken on Tetrahymena cultures treated with buffer (control) and with 10^{-8} M adrenomedullin (ADM), 10^{-8} M proadrenomedullin N-terminal 20 peptide (PAMP) and 10^{-8} M calcitonin gene-related peptide (CGRP) by applying NBD-phallacidin. (Microscope: Nikon Eclipse; binning: none; brightness: 1.1; gamma: 0.63; gain: 2.0; exposition: 10,000 ms; lens: 60×). **b** Fluorescent density of NBD-phallacidin measured by plate reader (LS50B, Perkin-Elmer; Exc 465 nm, Em 560 nm) as an index of F-actin content. Cells were treated by 10^{-12}, 10^{-10} and 10^{-8} M adrenomedullin (ADM), proadrenomedullin N-terminal 20 peptide (PAMP) and calcitonin gene-related peptide (CGRP) (*t* test: *y*: $p < 0.01$; *z*: $p < 0.001$)

study is only a short-term specificity, and that the neutral effect of CGRP could not select even a small sub-population with high chemotactic responsiveness. In contrast, the sub-populations selected with PAMP showed a significant decrease of Ch_{sel} coefficient (0.33 ± 0.02) (Fig. 6b). This result demonstrates that although the chemorepellent, PAMP, is a proper ligand to select small, chemotactically responsive sub-population, this positive relationship between the ligand and target cell is only acute and transient in the umpteenth sub-population. PAMP can elicit a characteristic chemorepellent response in the model cells. These results suggest that the chemorepellent nature of PAMP, as observed in the concentration range study, is a long-lasting characteristic in ligand-target cell interaction.

Concerning the responsiveness of selected sub-populations, it is necessary to discuss the altered responsiveness of cells selected with the control substance. In case of ADM and CGRP, the responsiveness of these groups was suppressed (C/ADM 83.79 ± 7.36 %and C/CGRP 82.47 ± 9.87) %. This is in contrast to PAMP, where a significant elevation was detected (C/PAMP 131.57 ± 13.34 %). These data, in accordance with other experiments [39], underline that the control substance—fresh control medium—itself possesses some significant signal. On the other hand, we should consider the differences in responsiveness of the CGRP-family peptides (ADM and CGRP) as well as PAMP.

We report here first that ADM and PAMP have chemotactic effects on eukaryotic unicellular target cells such as *Tetrahymena*. The parallels detected in the chemotactic behaviours of ADM and CGRP (particularly with respect to selection results) as well as the diverse effect of PAMP suggest that specific inductions of signalling mechanisms of the tested ligands are responsible for the generation of the corresponding chemotactic response. Known molecular structures support such potential mechanism diversities, as the presence of N-terminal ring structures of ADM and CGRP shows close relation to the biological efficacy of such molecules (in mammals), e.g. reverse responses and hypertensive effects were detected in ADM derivatives possessing truncated molecules without a ring structure [40]. We suppose, however, that for *Tetrahymena*, sequence similarities were important in the induction of a response; this is in contrast with PAMP which has no sequence homologies with either ADM or CGRP.

Considering the receptor(s) involved in the migration behaviour regulated by ADM, PAMP or CGRP and the triggered intracellular molecular mechanisms in Tetrahymena, authors call attention to the fact that chemotactic responses of Tetrahymena are elicited by a long list of ligands, nevertheless, only in 20–30 % of these molecules, it was possible to prove the receptor or receptor-like structures (e.g. insulin, endothelin-1) [31, 39] or the corresponding signalling mechanism (e.g. bradykinin, atrial natriuretic peptide) [32, 41]. The rest of the ligands have

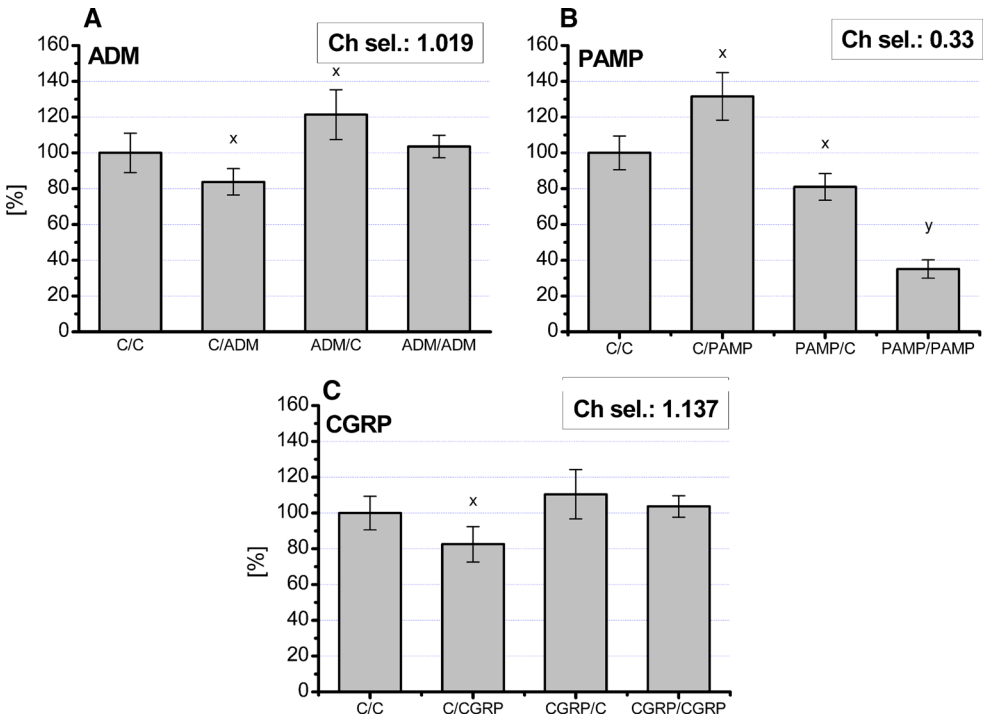

Fig. 6 Chemotactic selection of Tetrahymena pyriformis with adrenomedullin (ADM) (10^{-8} M), proadrenomedullin N-terminal 20 peptide (PAMP) (10^{-6} M) and calcitonin gene-related peptide (CGRP) (10^{-12} M)

chemoattractant or chemorepellent effect on the model cell, nevertheless, itself the receptorial component is still obscure.

Sensibility of the chemotactic responsiveness of our model Tetrahymena was proved in several experiments, e.g. amidation of the C-terminal of WSXWS peptides or mirror variants of Pro-X and X-Pro dipeptides—as a slight modification of physicochemical properties of the ligands—could also determine chemoattractant or chemorepellent effects elicited via surface membrane receptors of Tetrahymena [7, 42]. The above-mentioned results in concern with several others support the actually accepted theory of the eukaryote protozoa, which considers these functionally intact units of the surface membrane as "chemotaxis receptors" with ligand-dependent "chemoattractant" and "chemorepellent" modulator abilities [43].

In conclusion, our present experiments demonstrated that the vertebrate/human-type vasoactive molecule adrenomedullin (ADM) and its close relative compounds (PAMP and CGRP) have ligand-specific, diverse effects on the migratory behaviour of the eukaryotic ciliate *Tetrahymena pyriformis*. The complexity of swimming behaviour modulated by the three compounds underlines the significance to understand chemotaxis, chemokinesis and some characteristics of migratory behaviour (velocity, tortuosity) as an additive and complex functional unit realized on sub-population level. The results gained by chemotactic selection call attention that differences could be derived not only from structural differences of the ligands but also from the short-term (ADM, CGRP) or long-term (PAMP) character of the chemotaxis receptors induced by these peptides.

The results demonstrate that hormone-like substances, which have specific functions in mammals, can display different function at a unicellular level. Tetrahymena synthesizes and secretes many hormones which have mammalian characters. However, it is not known whether the hormones studied at present are synthesized by it. At the same time, these hormones influenced a basic function—chemotaxis—of Tetrahymena. This permits to suppose that receptors are present for them or for the sequences which are present in them. This supports the theory that the hormonal system of higher ranked animals can be deduced to a unicellular level [44, 45].

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