VIP stimulated enzyme activity in membranes prepared from Cl.19A cells in the range of concentration of 10^{-11}–10^{-8} M with an ED_{50} of 0.07 ± 0.02 nM VIP (fig. 5).

Discussion

Our finding in the Cl.19A cell line of a strong correlation between three parameters associated with VIP receptor-mediated biological events; namely, dissociation constant of receptor, ED_{50} for activation of adenylate cyclase and ED_{50} for increasing short circuit-current, clearly suggests that this cell line is a valid model for unraveling the cellular mechanisms involved in an important physiological function, i.e. intestinal secretion of electrolytes. The relevance to physiology of the studies performed on the Cl.19A cell line is further supported by the fact that the dissociation constant of the VIP receptor, its ability to discriminate between several VIP-related peptides, and its molecular size in these cultured human intestinal cells are the same as those previously reported in epithelial cells isolated from normal human colon[11,12,17,18].

Previous work has led to the isolation of several differentiated clonal derivatives from the undifferentiated HT29 cells, each of these clonal lines displaying distinctive features of intestinal differentiation. The function of ionic transepithelial transport is represented by Cl.19A cells[7], and the function of mucus secretion is typified by the Cl.16E cells[6]. It is worthwhile to emphasize the identical expression of VIP receptors in the 2 above-cited clonal cell lines (this paper and Laburthe et al.[10]). This includes dissociation constant and concentration of receptors, peptide specificity, molecular weight of the VIP receptors, and coupling to the adenylate cyclase system. Therefore it may be stated that VIP receptors are expressed in 2 lineages of intestinal differentiation, ending in cells with different functions, e.g., ion transport and mucus secretion. Whereas VIP stimulates chloride secretion in Cl.19A cells, it potentiates the cholinergic stimulation of mucus secretion in Cl.16E cells[10].

This work was supported in part by the Association Française de Lutte contre la Mucoviscidose (AFLM), by the Association pour la Recherche sur le Cancer (ARC), and the Ligue Nationale Française contre le Cancer. Dr. Grasset's present address is: Roussel Uclaf Nutrition, Tour Roussel Hoechst, 1 Terrasse Bellini, F-92800 Puteaux.

1 Laburthe, M., and Amiranoff, B., in: Handbook of Physiology, Neuroendocrinology of the Gut. Ed. G. M. Makhlouf. Am. physiol. Soc., Bethesda, MD, in press.
2 Grasset, E., Pinto, M., Dussaulx, E., Zweibaum, A., and Desjeux, J. F., Am. J. Physiol. *247* (Cell Physiol. *16*) (1984) C260.
3 Grasset, E., Bernabeu, J., and Pinto, M., Am. J. Physiol. *248* (Cell Physiol. *17*) (1985) C410.
4 Dharmsathaphorn, K., Mandel, K. G., Masui, H., and McRoberts, J. A., J. clin. Invest. *75* (1985) 462.
5 Zweibaum, A., Laburthe, M., Grasset, E., and Louvard, D., in: Handbook of Physiology. Intestinal Transport of the Gastrointestinal System. Eds M. Field and R. A. Frizzell. Am. physiol. Soc., Bethesda, MD, in press.
6 Augeron, C., and Laboisse, C. L., Cancer Res. *44* (1984) 3691.
7 Augeron, C., Maoret, J. J., Laboisse, C. L., and Grasset, E., in: Ion Gradient-Coupled Transport. Eds F. Alvarado and C. H. Van Os. Elsevier, Amsterdam 1986.
8 Fogh, J., and Trempe, G., in: Human Tumor Cells 'in vitro'. Ed. J. Fogh. Plenum Press, New York 1975.
9 Chen, T. R., Exp. Cell Res. *104* (1977) 255.
10 Laburthe, M., Augeron, C., Rouyer-Fessard, C., Roumagnac, I., Maoret, J. J., Grasset, E., and Laboisse, C., Am. J. Physiol. *256* (Gastrointest. Liver Physiol. *19*) (1989) G443.
11 Laburthe, M., Couvineau, A., and Rouyer-Fessard, C., Molec. Pharmac. *29* (1986) 23.
12 Couvineau, A., and Laburthe, M., J. clin. Endocr. Metab. *61* (1985) 50.
13 Laemmli, U. K., Nature *227* (1970) 680.
14 Bradford, M., Analyt. Biochem. *72* (1978) 248.
15 Laburthe, M., Bataille, D., and Rosselin, G., Acta endocr. *84* (1977) 588.
16 Laburthe, M., Rousset, M., Boissard, C., Chevalier, G., Zweibaum, A., and Rosselin, G., Proc. natl Acad. Sci. USA *75* (1978) 2772.
17 Broyart, J. P., Dupont, C., Laburthe, M., and Rosselin, G., J. clin. Endocr. Metab. *52* (1981) 715.
18 Laburthe, M., Amiranoff, B., Boige, N., Rouyer-Fessard, C., Tatemoto, K., and Moroder, L., FEBS Lett. *159* (1983) 89.

0014-4754/89/11-12/1102-04$1.50 + 0.20/0

The influence of in vitro sodium and potassium ion ratio on teleost melanosome intracellular motility

D. Burton

Ocean Sciences Centre (Marine Sciences Research Laboratory) and Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland (Canada A1C 5S7)
Received 17 January 1989; accepted 14 June 1989

Summary. The miniature neuroeffector system represented by the melanophore and neural elements of winter flounder (*Pseudopleuronectes americanus*) scale slips demonstrates asymmetrical sensitivity to progressive in vitro changes in the relative concentrations of Na^+ and K^+ ions. K^+ ions induce melanosome aggregation more readily than Na^+ ions evoke melanosome dispersion, reflecting the influence of K^+ induced depolarization on neurotransmitter release.
Key words. Melanophores; integumentary nerve plexus; sodium ions; potassium ions; neurotransmitter.

Since Spaeth's[1] studies on *Fundulus* scale-slip melanophores there has been increasing utilization of in vitro protocols in physiological and pharmacological experimentation on teleost chromatophores. Such protocols include the use of excised small areas of skin[2,3], split-fin preparations[4-9], scale slips[10-18] and chromatophores isolated by hydrolytic enzymes[19-21]. Some of these protocols have incorporated isotonic incubation media based on either Na^+ ions or K^+ ions, which respectively stimulate intracellular dispersion and aggregation of melanosomes. The purpose of the present communication is to describe melanosome motility in scale slips from winter flounder (*Pseudopleuronectes americanus*) in response to progressive changes in the ratio between Na^+ and K^+ ion concentrations in isotonic incubation media.

Materials and methods

Winter flounder (*Pseudopleuronectes americanus*) caught by scuba divers with hand nets off the Avalon Peninsula, Newfoundland, were maintained in stock tanks supplied with running seawater under seasonal photoperiod and temperature conditions. For the duration of the experiments flounder were kept singly in black 'plexiglas' aquaria (400 mm × 225 mm × 203 mm) supplied with running seawater, through a header tank, and illuminated continuously (60 W 1 m above). The aquaria were covered with wide mesh nylon net mounted on close-fitting wooden frames.

Scales, which can be plucked easily from the dark ocular side of flounder carry a small slip of skin posteriorly. Histological sections[22] demonstrate that such scale slips include dermal and epidermal tissue above the scale. Both dermal and epidermal melanophores are abundant and can be readily identified using morphological[23] and depth of focus criteria. Scale slips from the mid region of the integumentary pattern (i.e. from the general background component[24,25]) were immersed in physiological saline solution (PSS) for 15 min. The PSS had the following composition in mM/l: NaCl, 175.0; KCl, 2.7; $MgCl_2 \cdot 6\,H_2O$, 0.64; $CaCl_2$, 1.53; $NaHCO_3$, 5.0; glucose, 5.6. Scale slips were transferred to 'melanosome dispersing fluid' (DF) with the following composition in mM/l: NaCl, 177.7; $NaHCO_3$, 5.0; glucose, 5.6, or to 'melanosome aggregating fluid' (AF) with the following composition in mM/l: KCl, 177.7; $KHCO_3$, 5.0; glucose, 5.6. The pH (7.6–7.8) of all solutions was adjusted with 5% CO_2–95% O_2. Initial equilibration to either DF or AF was completed on a glass culture microslide (fluid, 0.35–0.4 ml) mounted on a thermal stage (Bailey Instruments, Model TS-2) and held on a microscope mechanical stage. After completion of this initial equilibration the scale slip melanophores were then equilibrated in stepwise sequences to each of the isotonic incubation media from solution 2 to solution 11 or, conversely, from solution 10 to solution 1 (table), composed of progressively changing proportions, by volume, of DF and AF. After each equilibration the incubation media were changed completely, but with their temperature maintained at 20 °C throughout the experiments.

Melanophore equilibration to each solution was recorded using melanophore index (MI) scales[26], in which 1 represents complete melanosome aggregation and 5 represents complete dispersion. Statistical analyses were performed by the Mann-Whitney U-test using the extended tables of Rohlf and Sokal[27].

Results

Winter flounder skin includes both dermal and epidermal melanophores[16]. Scale slips initially equilibrated to DF (table) and then to incubation medium 2, with Na^+ and K^+ concentrations (mM/l) in the proportion of 9:1, displayed extensive dermal melanosome dispersion (fig. 1) with a mean dermal MI of 4.44 ± 0.15 in both solutions. Equilibration to incubation medium 3 (table), with Na^+ and K^+ concentrations (mM/l) in the proportion of 8:2, resulted in considerable dermal melanosome aggregation (fig. 1), the mean dermal MI decreasing to 1.75 ± 0.16 which was statistically significant ($p > 0.001$). There was a further decline in dermal MI to 1.38 ± 0.18, which was not statistically significant ($p = 0.1$), in incubation medium 4 (table) with Na^+ and K^+ concentrations (mM/l) in the proportions of 7:3, and there were further smaller decreases in dermal MI as the proportion of K^+ relative to Na^+ was increased (fig. 1). In a second experiment scale slips initially equilibrated to AF (incubation medium 11, table) displayed a high degree of dermal melanosome aggregation (fig. 2), with a mean dermal MI of 1.2 ± 0.1. Progressive decreases in the concentration (mM/l) of K^+ relative to Na^+ to 3:7 did not result in any significant dermal melanosome dispersion, with dermal MI values remaining at 1.2 ± 0.1 to 1.3 ± 0.1: However, further decreases in K^+ concentration relative to Na^+ to 2:8 and 1:9 resulted in dermal MI increases to 1.94 ± 0.13 and 4.31 ± 0.23 respectively (fig. 2), both increases being statistically significant ($p < 0.005$ and $p < 0.001$). Finally, dispersing fluid (incubation medium 1, table) induced a further slight increase in dermal MI, which was not statistically significant ($p > 0.1$).

Proportions of DF and AF by volume, and the sodium and potassium salt concentrations in the incubation media

Medium	DF:AF	Na-K salt concentrations (mM/l) NaCl	$NaHCO_3$	KCl	$KHCO_3$
1(DF)	10:0	177.70	5.0	0	0
2	9:1	159.93	4.5	17.77	0.5
3	8:2	142.16	4.0	35.54	1.0
4	7:3	124.39	3.5	53.31	1.5
5	6:4	106.62	3.0	71.08	2.0
6	5:5	88.85	2.5	88.85	2.5
7	4:6	71.08	2.0	106.62	3.0
8	3:7	53.31	1.5	124.39	3.5
9	2:8	35.54	1.0	142.16	4.0
10	1:9	17.77	0.5	159.93	4.5
11(AF)	0:10	0	0	177.70	5.0

All incubation media also included glucose (5.6 mM/l).

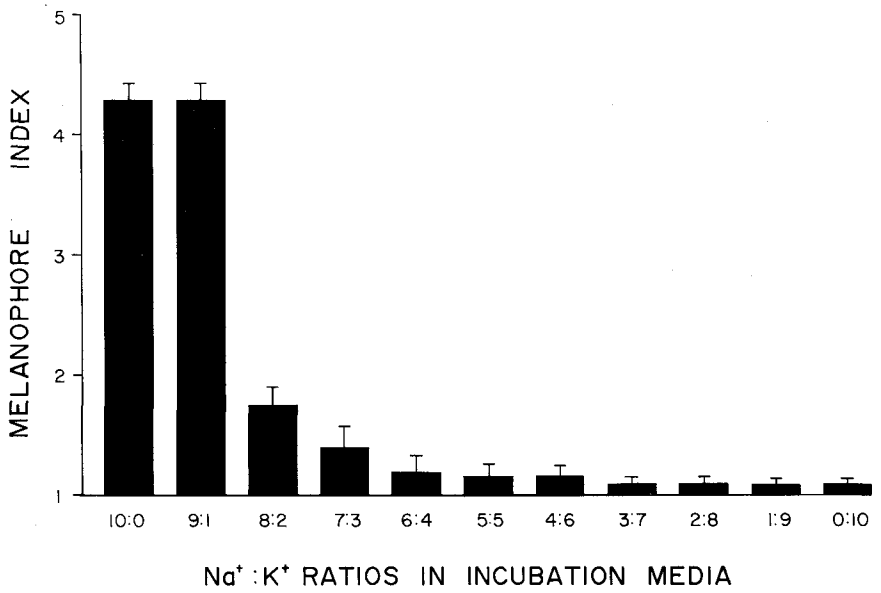

Figure 1. Dermal melanophore responses to progressive decreases in Na^+:K^+ (based on mM/l, table) following initial equilibration in DF. N = 8 scale slips.

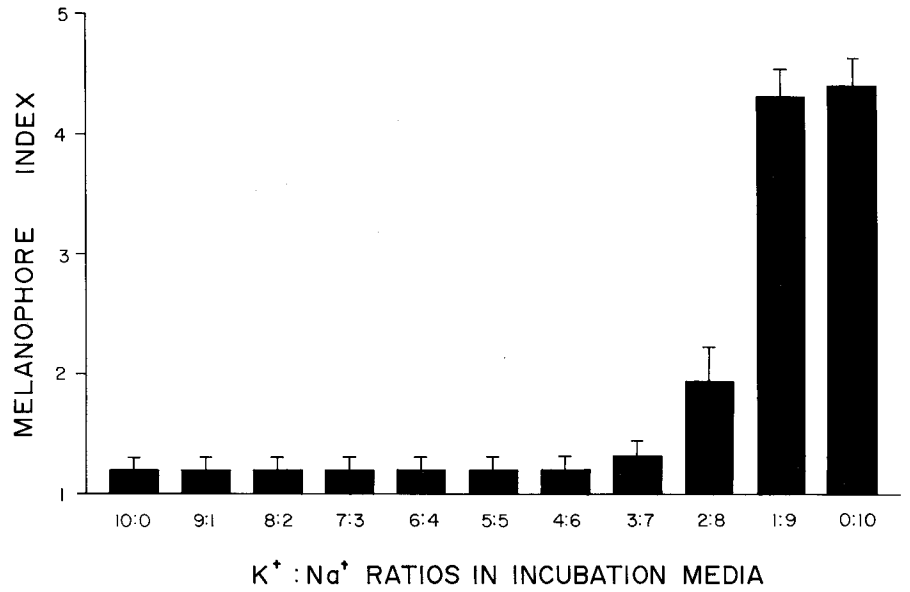

Figure 2. Dermal melanophore responses to progressive decreases in K^+:Na^+ ratio (based on mM/l, table) following initial equilibration in AF. N = 8 scale slips.

In both experiments the results for the epidermal melanophores were similar to those for the dermal melanophores. Irrespective of whether melanosomes were aggregated or dispersed initially, changes involving incubation media 2 and 3 (table) with concentrations (mM/l) of Na^+ relative to K^+ in the ratios of 9:1 and 8:2 were the most important, evoking the most extensive melanosome aggregation or dispersion in each experiment. Respectively, the Na^+ concentrations in these incubation media were 164.43 and 146.16 mM/l whereas the K^+ concentrations were only 18.27 and 36.54 mM/l (table).

Discussion

Essentially, flounder scale slips incorporate a miniature neuroeffector system consisting of melanophore and integumentary neural plexus elements. The present results demonstrate that in flounder this system has asymmetrical response characteristics to K^+ and Na^+ ion concentrations. As in *Fundulus*[1] K^+ ions are more potent in inducing melanosome aggregation than are Na^+ ions in eliciting melanosome dispersion. In their recent review Fujii and Oshima[28] consider the in vitro melanosome aggregating action of K^+ ions to be mediated through the release of neurotransmitter from the adrenergic neu-

rons innervating the melanophores. Fujii[29] states that very little is known about the melanosome dispersive action of Na^+ ions and the phenomenon has been disregarded since dispersed melanosomes have been equated with the resting state of melanophores. Fujii[29] also suggests that Na^+ ions may act by stimulating melanosome dispersing neurons, but Fernando and Grove[30] have disputed this interpretation. Neuromelanophore transmission and neural signal to melanosome motility transduction appear to be independent of Na^+ channels, in contrast with the neuronal component[28]. However, a possible relationship between membrane polarization and melanosome migration involving a Na^+ pump has been suggested[31]. K^+-rich AF and Na^+-rich DF represent means of clamping membrane polarity in the components of this physiological model and changes in K^+:Na^+ ratio in the incubation media will influence polarization. K^+ potency probably reflects a positive feedback in which a small degree of neuronal depolarization establishes an excitation threshold opening voltage sensitive Na^+ channels, and thereby triggering noradrenaline release. This would explain the almost complete melanosome aggregation in response to a relatively small increase in K^+. Thus, increasing K^+:Na^+ ratio effectively simulates the effect of electrical stimulation of scale slips of *Labrus ossifagus*[14] in inducing melanosome aggregation. In vivo dispersed melanosomes probably represent a 'resting' state of melanophores in relation to body fluids rich in Na^+ ions, irrespective of any additional melanosome dispersive neural and hormonal factors that may regulate these effectors.

Acknowledgments. This work was supported by an operating grant from the Natural Sciences and Engineering Research Council of Canada. Technical assistance was provided by summer student Ms C. Brennan and by Ms S. Kavanagh in preparing the graphs.

1 Spaeth, R. A., J. exp. Zool. *15* (1913) 527.
2 Healey, E. G., and Ross, D., Comp. Biochem. Physiol. *19* (1966) 545.
3 Fernando, M. M., and Grove, D. J., Comp. Biochem. Physiol. *48A* (1974) 723.
4 Fujii, R., and Miyashita, Y., Comp. Biochem. Physiol. *51C* (1975) 171.
5 Katayama, H., J. exp. Zool. *212* (1980) 21.
6 Kumazawa, T., and Fujii, R., Comp. Biochem. Physiol. *78C* (1984) 263.
7 Kumazawa, T., Oshima, N., Fujii, R., and Miyashita, Y., Comp. Biochem. Physiol. *78C* (1984) 1.
8 Miyashita, Y., Kumazawa, T., and Fujii, R., Comp. Biochem. Physiol. *77C* (1984) 205.
9 Kasukawa, H., Sugimoto, M., Oshima, N., and Fujii, R., Comp. Biochem. Physiol. *81C* (1985) 253.
10 Schliwa, M., and Bereiter-Hahn, J., Z. Zellforsch. *147* (1973) 107.
11 Rance, J., and Baker, B. I., Gen. comp. Endocr. *37* (1979) 64.
12 Visconti, M. A., and Castrucci, A. M. L., Comp. Biochem. Physiol. *70C* (1981) 293.
13 Visconti, M. A., and Castrucci, A. M. L., Comp. Biochem. Physiol. *82C* (1985) 501.
14 Andersson, R. G. G., Karlsson, J. O., and Grundström, N., Acta physiol. scand. *121* (1984) 173.
15 Morishita, F., Katayama, H., and Yamada, K., Comp. Biochem. Physiol. *81C*(1985) 279.
16 Burton, D., Comp. Biochem. Physiol. *87A* (1987) 699.
17 Burton, D., J. Fish Biol. *32* (1988) 433.
18 Burton, D., Comp. Biochem. Physiol. *90C* (1988) 263.
19 Schliwa, M., Yale J. Biol. Med. *50* (1977) Abst. 122.
20 Luby, K. J., and Porter, K. R., Cell *21* (1980) 13.
21 Clark, C. R., Taylor, J. D., and Tchen, T. T., In Vitro Cell devl Biol. *23* (1987) 417.
22 Burton, D., Experientia *34* (1978) 140.
23 Burton, D., Can. J. Zool. *57* (1979) 650.
24 Osborn, C. M., J. exp. Zool. *81* (1939) 479.
25 Burton, D., Proc. R. Soc. Lond. *B213* (1981) 217.
26 Hogben, L., and Slome, D., Proc. R. Soc. Lond. *B108* (1931) 10.
27 Rohlf, R. J., and Sokal, R. R., Statistical Tables, pp. 185–190. Freeman, New York 1981.
28 Fujii, R., and Oshima, N., Zool. Sci. *3* (1986) 13.
29 Fujii, R., J. Fac. Sci. Univ. Tokyo. Sec. IV *8* (1959) 371.
30 Fernando, M. M., and Grove, D. J., Comp. Biochem. Physiol. *48A* (1974) 711.
31 Castrucci, A. M. L., Comp. Biochem. Physiol. *50A* (1975) 453.

0014-4754/89/11-12/1105-04$1.50 + 0.20/0

Determination of cerebrospinal fluid and serum lead levels in patients with amyotrophic lateral sclerosis and other neurological diseases

E. Kapaki[1], J. Segditsa, Ch. Zournas, D. Xenos and C. Papageorgiou

Department of Neurology, Athens University, Aeginition Hospital, 74 Vass. Sofias Ave., GR-115 28 Athens (Greece)
Received 20 February 1989; accepted 30 May 1989

Summary. In a total of 62 samples of cerebrospinal fluid (CSF) and an equal number of serum samples obtained from 16 patients suffering from amyotrophic lateral sclerosis, 22 patients suffering from miscellaneous neurological diseases, and 24 controls, lead was measured by atomic absorption spectrophotometry. No statistical difference in lead concentration was found between the above three groups.
Key words. Lead; cerebrospinal fluid (CSF); amyotrophic lateral sclerosis (ALS).

The lead (Pb) level in the cerebrospinal fluid (CSF) in neurological disorders, mainly in amyotrophic lateral sclerosis (ALS), has already been studied[2–7]. However, the reported results are uncertain and often contradictory; this is probably due to methodological errors. The purpose of this paper is to present our results for CSF and serum Pb content in patients suffering from ALS compared with other neurological diseases and controls.