hearts of patients who suffer from coronary diseases[30]. Vitamin D stimulates enzymes like glutamate-transaminases that are involved in glutamate metabolism in the skin[31]. Such a possible mechanism in the heart needs further investigation. Together our data from combined histochemical and physiological studies indicate that 1,25-D_3 has direct genomic actions on cardiomyocytes predominantly in the right atrium that result in changes of manufacture and secretion of ANF. Other simultaneous effects on glutamate turnover, ionic concentrations, and conductivity are likely. Simultaneous cooperative or antagonistic genomic actions of sex and adrenal steroids[4,17–19,32,33] on ANF turnover must be considered and viewed interactively with those of vitamin D. As in other tissues, the role of vitamin D – soltriol is likely to adjust cardiovascular and other vital functions to the seasonal changes to optimize survival and reproduction[4,6].

* To whom all correspondence should be addressed.

1 Neer, R. M., Davis, T. R. A., Walcott, A., Koski, S., Schipis, P., Taylor, I., Thorington, L., and Wurtman, R. J., Nature *229* (1971) 255.
2 Norman, A. W., (Ed) Vitamin D. The Calcium Homoeostatic Steroid Hormone. Academic Press, New York 1979.
3 Stumpf, W. E., Histochemistry *89* (1988) 209.
4 Stumpf, W. E., Experientia *46* (1990) 13.
5 Stumpf, W. E., and O'Brien, L. P., Histochemistry *87* (1987) 393.
6 Stumpf, W. E., and Denny, M. E., Am. J. Obstet. Gynec. *161* (1989) 1375.
7 Stumpf, W. E., and Privette, T. H., Psychopharmacology *97* (1989) 285.
8 Stumpf, W. E., Sar, M., and DeLuca, H. F., in: Hormonal Control of Calcium Metabolism, p. 222. Eds D. V. Cohn, R. V. Talmage and J. L. Matthew. Exerpta Medica, Amsterdam 1981.
9 Stumpf, W. E., Sar, M., Reid, F. A., Tanaka, Y., and DeLuca, H. F., Science *206* (1979) 1188.
10 Walters, M. R., Wicker, D. C., and Riggle, P. C., J. molec. cell. Cardiol. *18* (1983) 67.
11 Genest, J., and Cantin, M., Rev. Physiol. Biochem. Pharmac. *110* (1988) 1.
12 Stumpf, W. E., in: Methods in Cell Biology, vol. XIII, p. 171. Ed. D. M. Prescot. Academic Press, New York 1976.
13 Stumpf, W. E., and Duncan, G. E., in: Methods of Neuroscience, vol. 3, p. 35. Ed. P. M. Conn. Academic Press, New York 1990.
14 Gutkowska, J., Horky, K., Schiffrin, E. L., Thibault, G., Garcia, R., De Lean, A., Hamet, P., Tremblay, J., Anand-Srivastava, M. B., Januszewicz, P., Genest, J., and Cantin, M., Fedn Proc. *45* (1986) 2101.
15 Gutkowska, J., Genest, J., Thibault, G., Garcia, R., Larochelle, P., Cusson, J. R., Kuchel, O., Hamet, P., de Lean, A., and Cantin, M., Endocr. Metab. Clin. North Am. *16* (1987) 183.
16 Steinhelper, M. E., Cochrane, K. L., and Field, L. J., Hypertension *16* (1990) 301.
17 Stumpf, W. E., Sar, M., and Aumüller, G., Science *196* (1977) 319.
18 Stumpf, W. E., Back, H., and Forssmann, W.-G., in: Functional Morphology of the Heart, p. 95. Eds W.-G. Forssmann, D. M. Scheuermann and J. Alt. Springer-Verlag, New York 1989.
19 Cantin, M., Biochem. biophys. Res. Commun. *131* (1985) 806.
20 Sar, M., Miller, W. L., and Stumpf, W. E., Physiologist *24* (1981) 70.
21 Törnquist, K., and Lanberg-Allardt, C., in: Vitamin D. A Chemical Biochemical and Clinical Update, p. 363. Eds A. W. Norman, K. Schaefer, H. G. Grigoleit and V. D. Herrath. Walter de Gruyter Verl., Berlin 1985.
22 Clark, S. A., Stumpf, W. E., and Sar, M., Diabetes *30* (1981) 382.
23 Clark, S. A., Stumpf, W. E., Sar, M., and DeLuca, H. F., Am. J. Physiol. *253* (1987) E 99.
24 Weishaar, R. E., and Simpson, R. U., J. clin. Invest. *79* (1987) 1706.
25 Ackermann, U., Fedn Proc. *45* (1986) 2111.
26 Ertl, G., and Bauer, B., Eur. Heart J. *11* (suppl. B) (1990) 53.
27 Parkes, D. G., Coghlan, J. P., McDougall, J. G., and Scoggins, B. A., Am. J. Physiol. *254* (1988) H 811.
28 Blaine, E. H., Seymour, A. A., Marsh, E. A., and Napier, M. A., Fedn Proc. *45* (1986) 2122.
29 Bittl, J. A., and Shine, K. I., Am. J. Physiol. *245* (1983) H 406.
30 Thomassen, A. R., Nielsen, T. T., Bagger, J. P., and Henningsen, P., Clin. Sci. *64* (1983) 33.
31 Holick, M. F., Smith, E., and Pincus, S., Arch. Dermat. *123* (1987) 1677a.
32 St-Louis, J., Parent, A., Gutkowska, J., and Schiffrin, E. L. Am. J. Physiol. *254* (1988) H 1027.
33 Riegger, A. J. G., Eur. Heart J. *11* (suppl. B) (1990) 79.

0014-4754/91/090958-05$1.50 + 0.20/0

Reevaluation of hydropathy profiles of voltage-gated ionic channels

A. Sawaryn* and H. Drouin[a]

** Institut für Physik and [a] Institut für Physiologie, Medizinische Universität zu Lübeck, Ratzeburger Allee 160, D-2400 Lübeck 1 (Germany)*

Abstract. A reevaluation of the secondary structure of Na, Ca and K channel proteins led to the following results. Only three segments (S1, S5 and S6) of each repeat are sufficiently hydrophobic to be predicted as transmembrane helices, if a window of 19 amino acids is used. Some of the S2 and S3 segments show higher hydrophobic values when calculated with the window of 9 amino acids and can be predicted as short helices. S4 segments are strongly hydrophilic and cannot be predicted as transmembrane helices. Some of the S2, S3 and S4 segments have an amphipathic character; however, these helices do not span a membrane. A model is proposed where 12 hydrophobic transmembrane helices surround 12 shorter helices, forming a hydrophilic pore. In addition, a unique pattern for S4 segments of voltage-gated channel proteins is defined.
Key words. Voltage-gated ionic channels; Na, Ca, K channel proteins; hydropathy profiles; secondary structure; sequence pattern of segment S4.

The primary and secondary structures of voltage-gated ionic channel proteins have been the topic of numerous studies. The amino acid sequences of several proteins forming channels for Na, Ca and K ions are now available (see SWISS-PROT Protein Sequence Database, Release 14). The current model for the secondary structure of these channel proteins was predicted on the basis of hydropathy profiles as calculated by the method of Kyte and Doolittle[1]. Six transmembrane helices (S1, S2, S3, S4, S5 and S6) are assumed to be present within each repeat, and altogether 24 transmembrane helices in four repeats are thought to form one ionic channel with a hydrophilic inner wall[2]. Alternative models of voltage-gated channels have also been proposed, where an even higher number of helices is predicted[3,4] or, on the other hand, only a single channel-helix with full functionality is postulated[5].

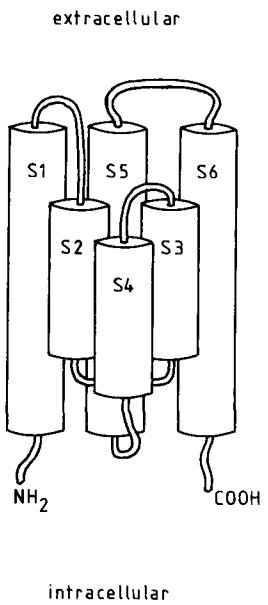
The interpretation of hydropathy profiles, which led to the current model with six transmembrane helices in one repeat, appears not to be straightforward. Lately it has been shown that the very popular hydrophobicity scale according to Kyte and Doolittle does not give the best predictions for the structures of membrane proteins[6]. Therefore, we favor another hydrophobicity scale proposed by Engelman et al.[7] (GES method); this scale is based on the free energies of transfer of amino acid side chains in α-helical polypeptides from water to oil. We applied the GES method for the prediction of helices in the subunits of the photosynthetic reaction center from *Rhodopseudomonas viridis*, and the results obtained were even better than those reported by Fasman and Gilbert[6] for other methods.

Hydropathy analysis of segments of voltage-gated ionic channel proteins using GES and Jähnig's methods

Repeat of channel protein		S1	S2	S3	S4	S5	S6
Na	(eel)						
	I	H	A	h	A	H	H
	II	h	h/A	h/A	n	H	H
	III	H	A	h	n	H	H
	IV	H	h	h	A	H	H
Na I	(rat)						
	I	H	n	h	n	H	H
	II	H	h/A	h	n	H	H
	III	H	n	n	n	H	H
	IV	H	h/A	H	n	H	H
Na II	(rat)						
	I	H	A	h	A	H	H
	II	H	h/A	h	n	H	H
	III	H	A	A	n	H	H
	IV	H	h/A	H	A	H	H
Ca	I	H	h	h	A	H	H
	II	H	A	h	A	H	H
	III	H	h	h	n	H	H
	IV	h	h/A	h/A	n	H	H
K	I	H	h	h	n	H	H

H, highly hydrophobic segment within window of 19 amino acids, predicted as transmembrane helix (GES method[7]); h, highly hydrophobic segment within window of 9 amino acids, predicted as short helix (GES method[7]); A, amphipathic helix predicted according to Jähnig's method[8,9]; n, neither H, h nor A.

The amino acid sequences are taken from the following sources: Na (eel)[14], Na (rat) I[2], Na (rat) II[2], Ca[15], K[16].



Model for one repeat of voltage-gated channel

In addition, membrane-spanning helices could have an amphipathic character, and this feature should be taken into account in proposing models of voltage-gated channel proteins. Jähnig[8,9] developed a special algorithm for the calculation of amphipathy profiles, which was successfully applied in studies on membrane proteins. These considerations prompted us to reevaluate the predictions of the secondary structures of several voltage-gated channel proteins using hydropathy and amphipathy analyses.

The results presented in the table indicate that only three segments (S1, S5 and S6) of each repeat are sufficiently hydrophobic to be predicted as transmembrane helices, if the window of 19 amino acids is used. Some of the S2 and S3 segments show higher hydrophobicity values when calculated with the window of 9 amino acids instead of 19 and, therefore, can be predicted as short helices. The possible occurrence of short helices in channel structures was discussed by Lodish[10]. S4 segments are strongly hydrophilic in all cases studied here and cannot be predicted as transmembrane helices according to hydropathy analysis. However, if the analysis were made using the hydrophobicity scale by Kyte and Doolittle, few S2 and S3 segments could be predicted as transmembrane helices, but all S4 segments would remain hydrophilic in accordance with the results of the GES method. The amphipathy analysis as proposed by Jähnig reveals that some of the segments S2, S3 and S4 have an amphipathic character. However, the lengths of the amphipathic domains are insufficient to span a membrane. Our results lead to the model shown in the figure.

Four repeats are thought to form one ionic channel with a hydrophilic inner wall. In our model, segment S4 is located near the pore region and, therefore, its structure and its function deserve special attention.

The primary structure of segment S4 includes 4 to 8 positively charged amino acids (arginine, lysine) which are regularly arranged in the sequence, each at every third position. This structure is highly conserved in voltage-gated channel proteins. However, searching for such a sequence pattern in the whole protein sequence database (SWISS-PROT) reveals that it is not characteristic for channel proteins. The unique pattern for S4 segments of voltage-gated channel proteins, as found according to the rules outlined by Bairoch[11], is defined as follows:

R-X-[FILV]-R-[LVAI]-[LVAI]-R-X-X-[RK].

This characteristic pattern of segment S4 consists of 10 amino acids. It contains 4 charged amino acids (arginine, lysine) and some strongly hydrophobic amino acids between them. This pattern appears in all the 40 known S4 segments; only one, the potassium channel protein, DRK1, has a methionine in position 3 of the pattern. There is one non-channel protein, cystatine, with such a pattern (data bank entry: CYTC$BOVIN).

S4 segments from repeats I and II of Na-II-channel proteins were the objects of extensive studies involving the combined use of site-directed mutagenesis and patch-clamp recordings[12]. These investigations provided evidence that the positive charges in S4 segments are involved in the voltage-sensing mechanism for activating of the channel. We calculated hydrophobicity values, using the window of 13 amino acids, for the wild-type and for mutants of segment S4. We have found that the substitutions of amino acids did not lead to significant increases of hydropathy values. It appears that rearrangements of the secondary structure are not responsible for the electrophysiological effects observed in this case. Lately Auld et al.[13] observed a dramatic change in the gating properties of voltage-dependent Na-II-channel protein, after replacement of a neutral amino acid (phenylalanine in place of leucine) in segment S4 of repeat II. This finding can be explained as being due to conformational changes, but only a minor increase of the hydrophobicity value was found in this case. Thus small conformational alterations cannot be excluded, and both the charge and the conformation of S4 segments have to be considered in attempts to elucidate the voltage-dependent gating mechanism of channel proteins.

1 Kyte, J., and Doolittle, R. F., J. molec. Biol. *157* (1982) 105.
2 Noda, M., Ikeda, T., Kayano, T., Suzuki, H., Takeshima, H., Kurasaki, M., Takahashi, H., and Numa, S., Nature *320* (1986) 188.
3 Guy, H. R., Curr. Top. Membr. Transport *33* (1988) 289.
4 Guy, H. R., and Conti, F., Trends Neurosci. *13* (1990) 201.
5 Benndorf, K., Eur. Biophys. J. *17* (1989) 257.
6 Fasman, G. D., and Gilbert, W. A., Trends biochem. Sci. *15* (1990) 89.
7 Engelman, D. M., Steitz, T. A., and Goldman, A., A. Rev. Biophys. biophys. Chem. *15* (1986) 321.
8 Jähnig, F., in: Prediction of Protein Structure and the Principles of Protein Conformation, pp. 707–717. Ed. G. D. Fasman. Plenum Press, New York 1989.
9 Jähnig, F., Trends biochem. Sci. *15* (1990) 93.
10 Lodish, H. F., Trends biochem. Sci. *13* (1988) 332.
11 Bairoch, A., PROSITE: A Dictionary of Protein Sites and Patterns, University Geneva, Fifth Release, 1990.
12 Stühmer, W., Conti, F., Suzuki, H., Wang, X., Noda, M., Yahagi, N., Kubo, H., and Numa, S., Nature *339* (1989) 597.
13 Auld, V. J., Goldin, A. L., Krafte, D. S., Catterall, W. A., Lester, H. A., Davidson, N., and Dunn, R. J., Proc. natl Acad. Sci. USA *87* (1990) 323.
14 Noda, M., Shimizu, S., Tanabe, T., Takai, T., Kayano, T., Ikeda, T., Takahashi, H., Nakayama, H., Kanaoka, Y., Minamino, N., Kangawa, K., Matsuo, H., Raftery, M. A., Hirose, T., Inayama, S., Hayashida, H., Miyata, T., and Numa, S., Nature *312* (1984) 121.
15 Tanabe, T., Takeshima, H., Mikami, A., Flockerzi, V., Takahashi, H., Kangawa, K., Kojima, M., Matsuo, H., Hirose, T., and Numa, S., Nature *328* (1987) 313.
16 Pongs, O., Kecskemethy, N., Müller, R., Krah-Jentgens, I., Baumann, A., Klitz, H. H., Canal, I., Llamazares, S., and Ferrus, A., EMBO J. *7* (1988) 1087.

0014-4754/91/090962-03$1.50 + 0.20/0

Some extracellular matrix elements as markers of 'capillary tunnels' in hypertrophied rat heart

A. Ratajska, Z. Gawlik and E. Fiejka

Department of Pathological Anatomy, Institute of Biostructure, Medical Academy of Warsaw, Chalubińskiego 5, PL-02-004 Warsaw (Poland)
Received 5 October 1990; accepted 15 February 1991

Abstract. We studied the distribution of the extracellular matrix proteins fibronectin (FN) and laminin (LM) in the hypertrophied hearts of spontaneously hypertensive rats (SHR), using an immunofluorescence method with specific antibodies. The immunohistochemical reaction was positive in the cytoplasm of some hypertrophied cardiomyocytes. The results showed that FN and LM can be used as markers for tunnels, i.e. intracardiocytic invaginations of the sarcolemma. The tunnels observed contained capillaries.
Key words. Heart hypertrophy; spontaneously hypertensive rats; tunnel capillaries; fibronectin; laminin; immunohistochemical staining.