ELSEVIER

Cell Calcium xxx (2004) xxx–xxx

cell calcium

www.elsevier.com/locate/ceca

Review

A cell physiologist between East and West of Germany

Gerrit Isenberg*

Department of Physiology, Julius-Bernstein-Insitut für Physiologie, Martin-Luther-University Halle, Halle 06097, Germany

Received 1 December 2003; accepted 12 January 2004

I was born in 1942 in the small city of Halberstadt. My father was a surgeon and owned a small private clinic, he ended up in jail in 1943 when he said something critical about the Nazi regime. In April 1945, Halberstadt was bombed flat. My mother fled with me as a little child into the rocks surrounding the city. In May 1945, the Nazi regime ended and my father was released as a sick and aged person. Essentially, I grew up under the love of my grandmother. I had a childhood very much like other children in those post war days in Leipzig, a medium-sized city in the "wrong" eastern part of Germany, the GDR (German Democratic Republic).

At the age of 16, I spent my summer vacations with the Ramshorn family. The Ramshorns lived in the countryside in an Institute of the German Academy where father Ramshorn was a Professor of plant physiology. I enjoyed the family with daughter Elisabeth and son Reinhard, but I was excited by the father who introduced me to the laboratory: using a stopwatch, I observed how seedlings changed their colour from white to green on exposure to light. As far as I can remember, this was my first contact with science, and it impressed me persistently. After finishing school in 1960, I had to decide what to do next. Could I study physiology? Professor Ramshorn pointed out to me that a solid professional background is important for survival during difficult times. I accepted his advice to postpone the decision of whether or not to do science and the kind of science, till the time I would complete my education as a "normal physician". Despite facing problems due to not belonging to a working class family, I entered the Karl-Marx-University Leipzig in October 1960 as a student of medicine.

1. Student life (Leipzig 1960–1966)

The first years in university were fun. I liked the lectures and the practical courses, and the first state examination (Physikum) yielded the best grades. Since I liked human physiology best of all the subjects in the pre-clinical course, I decided to become a physiologist not at the end but in the middle of my studies. I wished to become a physiologist because I was naively interested in the functioning of the human body. I was convinced that my medical education was not enough to understand physiology and therefore struggled for a permit to study Physics in combination with Medicine. When this was allowed by the central government, I began to study Physics in October 1963. Student life was fun as girls and boys lived together. I married in 1964, and shortly had a young family of two sons, Jörg 1965 and Dirk 1966. The communist system supported young families like ours by offering Kindergartens. The two grandmothers were in the mid fifties; although they were occupied by their professions, they could always find a way to handle the children with love when the Kindergarden could not take the children because of diarrhoea, severe cough, etc.

In the German university, medical students work for their thesis (MD) before their final examination, and do this work in the evenings or weekends. I found my adviser in Prof.

* Tel.: +49-345-557-1886; fax: +49-345-557-4019.
E-mail address: gerrit.Isenberg@medizin.uni-halle.desenescent (G. Isenberg).

0143-4160/$ – see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.ceca.2004.01.005

Georg Küchler, Physiology. He asked me to measure membrane potentials of frog skeletal muscle fibres as they were modulated by changes in extracellular pH and [Ca^{2+}]. For most of the time I put together valves left over from the war times into a "cathode follower" that was used for the actual measurements. The thesis was defended in 1966 and I received the title of an MD at the time when I had finished the final state examination as a physician.

2. Early university life (Leipzig 1966–1972)

As a young physician, I found my place working on muscle physiology in Georg Küchler's group. We were a team of 4–5 scientists, and I was introduced to the method of voltage clamping isolated skeletal muscle fibres by Merrem who had developed a double sucrose gap [1]. I enjoyed the struggle to prepare a single muscle fibre under the stereomicroscope with forceps and scissors, cutting down the frog semitendinosus muscle ideally to a single cell but unfortunately sometimes to no cell! I did not so much enjoy the struggle with the equipment, particularly the home-made amplifiers. In addition, there were the problems of how to measure the recorded membrane currents from a photograph taken from the screen of the oscilloscope. On the whole, however, it was a great fun.

2.1. Problems of young scientists

I have often been asked to explain why my scientific output was so low in the former GDR. The most important factors were (1) lack of equipment, (2) lack of scientific interactions, and (3) struggle with political indoctrination. The first point is easy to understand: the former GDR always had financial problems in terms of "hard currency". Electrophysiology was nearly impossible without adequate equipment, and a lot of time was spent (wasted) on making the equipment rather than carrying out scientific experiments. The experience of having no way to compete with the leading labs in the world eventually depresses the spirit, and building home-made equipment replaces science as the purpose of the lab. Missing chemicals made some experiments impossible; in my case, there was no chance to test with TTX whether the measured membrane currents were the classical Na^+ currents indeed [2]. Absence of less sophisticated chemicals such as HEPES buffer or sucrose free of contaminating salt was a problem as well.

2.2. Scientific isolation

A young scientist should leave his own lab, study the world for new ideas, techniques and eventually, should change over to new fields. Generally, foreign travel did not exist in the former GDR. Beside money restrictions, the communist party (SED) was afraid that one would not return, or tell bad stories on the situation in the country. With the exception of a small number of conformist people, the normal scientist remained isolated. How can one learn that the most interesting experiment had been already done, but not published because of negative results? How could I get feedback in order to find out whether my experiments were nonsense or worth continuing? Manuscripts were difficult to publish. We were obliged to send them to a peculiar GDR-owned journal, and the answer was frequently "not this year, we have consumed all the printing paper". Contact with international journals was forbidden, if it occurred nevertheless, the manuscript was smuggled out of the country. In 1970, the youngsters in the department were asked to work for a year in the Soviet Union. As an electrophysiogist, I admired the work published by Kostyuk's group [3], and I applied for a 1-year stay in Kiev. The result was negative, the SED decided that I was "unworthy for such an award". In a similar way, I could not obtain the visa necessary to participate the meeting of the Biophysical Society, held in 1972 in Moscow.

2.3. Indoctrination

"Wer nicht für uns ist, ist gegen uns" (who does not support us is against us, as famous Bolshevik and novelist Maxim Gorki used to say) was written in huge white letters on a red flag that hung in front of the department building. We had the tendency to forget this wisdom when the sign was out of sight. However, it applied to the daily university life. Every Monday morning we had an official brainwashing. The SED members in the department expected us to "positively" participate in the discussions, to express verbally support and sympathy for the official politics. We were afraid that a wrong answer would identify us as an "enemy of the working class" with the consequence of being fired. As a result, most of us developed some type of split brain: one part was responsible for the official answers, the other considered these answers as lies. We were trained how to work with both parts simultaneously. Working with a split brain applied also to private life. As was the case for many people, we found a niche between all the demands of the state, a small quiet place with family, friends and music as a hobby. However, we did not know who could be trusted not to report political conversations to the secret service (Stasi), and we assumed that the SED had installed bugs in our flat.

2.4. The escape (October 1972)

As an young assistant, I had to join medical students in October 1971 and 1972 when they picked up potatoes from the ground. We had done this ourselves before, and I still felt like one of the students. However, the assistant was not sent to do the students' job, he was supposed to be a supervisor. It was less important to organise the work (this was usually done by the farmers) than to control the students' discussions and political jokes. I was not aware that one of the students wrote down what I did or rather what I did not

do, and this Stasi report destroyed my budding career. In August 1972, I was fired. I tried to find another post at the Technical University Ilmenau. The dean from Ilmenau came to Leipzig to read Isenberg's cadre paper. This contained the judgement "Isenberg cannot be trusted to educate students in our sense". With such a comment, the dean told me, no East German university would employ me.

When the hope for a scientific future had disappeared and the frustration with the communist system had become unbearable, I heard about the small chance to escape through the Iron Curtain. My West German friends had contacted a professional escape organization who would transport the family with the two children for 60,000 West DM. My friends lent us this money, and came to Leipzig and East Berlin to tell us what to do. We were told to meet a pickup at 10 p.m. in a forest near the transit highway between West Germany and West Berlin. Still in East Germany, we entered a prepared truck through a secret door, the official door being sealed. The truck with us inside went back to the transit highway and rolled towards West Berlin. At the border, the engine had to be switched off, and my younger son was starting to cry, despite all the pre-medications we had given him. I had to cuddle him for what felt like hours. Finally, at around midnight of 22 October, we were in West Berlin.

3. As a young assistant in West Germany (Homburg 1972–1985)

In West Germany, we needed two jobs to raise money, not only for living but also to pay back the debts of 60,000 DM. By calling up we got some idea which department might have vacancies and should be visited. I started these visits in Homburg, found a job as a Professor, Wolfgang Trautweins Department, and we settled down there. The city was small, and the university community helped us "immigrants from East Germany" by clothing and furniture. The children were alone until 5 p.m. with the keys in their hands, but were safe because the neighbours watched them. During the initial 6 months, I felt like a stranger, there were so many things I did not know. I had problems with open discussions and had to learn that I was expected to express my own opinion. For a long time, I did not dare to say "I did wrong" for fear of the consequences. My colleagues were amused watching me to hoard old equipment in the lab, independent of whether or not I could use it. After 6 months I felt at home.

3.1. Electrophysiology on cardiac Purkinje fibres

The first problem I was given by Wolfgang Trautwein was to search for the existence and to study the properties of a current generated by active sodium transport. This was not the problem I had dreamt of, but I tried. A Purkinje fibre from the sheep heart is a white strip, composed mostly of connective tissue. Invisibly, somewhere within the interior, it carries a cord of Purkinje cells. As early as 1964, Wolfgang Trautwein had voltage clamped this tissue with two microelectrodes [4]. He showed me how to impale the cells, however, he was unable to pass over the information of how he found them. "You poke through the connective tissue as you force the fork through the skin of a sausage". This easy task took quite a while to learn. I used dihydroouabain to block the pump activity and could define the different currents as pump current. The final paper [5] was well accepted throughout the world and gave me the chance to present the data in 1974 at a Gordon conference.

The times in Homburg were not always golden. In 1979, my contract as Assistant Professor ended. When I tried to find a possible way out, there was none. The German university system had a bad experience; until 1973 everybody with a second degree (habilitation) got a permanent position. Since a lot of good jobs were occupied by not so good scientists, a new law was made that ended the position of an Assistant Professor after 5 years irreversibly, and my habilitation in 1976 did not make it any better. In that miserable situation, friends with similar problems emigrated to USA. The German Research Council (Deutsche Forschungsgemeinschaft, DFG) solved the problem and introduced the Heisenberg fellowship, the award that supported young scientists. The fellowship lasted 5 years, without teaching obligations, and encouraged me to perform research in the labs of Eduard Carmeliet (Leuven, Belgium [6]), John Blinks (Rochester, MN), Arthur M. Brown (Galveston, TX [7]) and Emilio Kabela (Mexico City, Mexico).

3.2. The first $[Ca^{2+}]_c$ measurements

In 1975, I was taught by Hans Dieter Lux, to make Ca^{2+} sensitive microelectrodes, and I used them to measure $[Ca^{2+}]_c$ in Purkinje fibres. Together with Gerhard Dahl from the department of physiology, the $[Ca^{2+}]_c$ measurements were applied to the problem of electrical coupling between the cells. I was fascinated by Gerhard's freeze-fracture electron micrographs that showed the connexins as particles. We were impressed that we could measure changes in particle diameter and height that seemed to correlate with the increase of $[Ca^{2+}]_c$ and cell-to-cell resistance [8]. In my dreams, I was fascinated by the possible link of structural and functional changes.

The Ca^{2+} sensitive electrodes had a response time of the order of minutes, too slow to address the question of Ca^{2+} modulation of K^+- and Ca^{2+} currents. Therefore, I used my Heisenberg award to visit the Mayo Foundation in Rochester (MN), the "Roma" of Ca^{2+} measurements with aequorin. John Blinks accepted me kindly and put me together with Gil Wier. Gil had already injected aequorin into Purkinje fibres to analyse Ca^{2+} transients produced by action potentials, and he had described two components. During my visit we combined these studies with the voltage clamp. The results suggested that the initial fast component would originate from Ca^{2+} influx via the Ca^{2+} inward current, and that the slow component would be generated by Ca^{2+} release from the SR

[9]. I had a wonderful time in Rochester. The small town surrounding the Mayo Foundation provided a highly intellectual atmosphere with many discussions. I always felt safe, even if I returned to bed at 1 a.m. I had similar experiences when I worked as a guest in Leuven, Galveston and Mexico City.

3.3. Isolated ventricular myocytes

In Homburg Department of Physiology I was chaired by Robert Stämpfli who analysed sodium currents at the Ranvier node. Stämpfli respected my work, however, with a lot of scepticism. He did not believe that one can voltage clamp multicellular preparations such as a Purkinje fibres or ventricular trabeculae. He suspected that the current would not flow in the way we would like it to. This was also true for multicellular papillary muscles; Wolfgang Trautwein was never satisfied when the voltage clamped them with the sucrose gap [10]. Another type of critical comment came from Alex Fabiato, saying that our Ca^{2+} transients were reporting signals from cells that were poisoned by the injected aequorin and would not contribute to contraction and membrane currents. Finally, we were aware that the clamp currents were changing their driving force; with Clive Baumgarten from Chicago I had shown that the potassium concentration in the small extracellular space between the cells changes during the clamp pulse [11]. Thus, I dreamt of avoiding all these artefacts by applying voltage clamp experiments to isolated cells.

The first trials on Purkinje fibres remained unsuccessful for a year. I squeezed tissue out of the Purkinje fibre and treated the mass with collagenase. Subsequent microscopy revealed only debris of broken cells. In the following year, I shared these trials with my student Udo Klöckner. We changed rat hearts that were retrogradely perfused with Ca^{2+} free collagenase containing solutions; Trevor Powell in Oxford had shown that this method can deliver myocytes that were "normal" in such a sense that Na^+ currents could be recorded [12]. However, re-introduction of physiological 1.8 mM Ca^{2+} killed the isolated cells; the cells contracted spontaneously, had problems in relaxing, rounded up and died within 3 min.

How to overcome this so-called "Ca^{2+} paradox"? Speculatively, we separated the cell isolation procedure into two periods: during the first period the tissue would dissociate into leaky cells, and during the second period the ion concentrations in the cytosol would equilibrate with those in the extracellular space because the pumps were exhausted in compensating for the leak. The final exposure to mM concentration of Ca^{2+}, necessary to repair the membrane damage, would kill the cells that were unable to handle the excessive Ca^{2+} influx. Udo followed up the concept that the Ca^{2+} paradox could be avoided by modifying the cytosol during the period of leakiness. In the Ca^{2+}-free medium he substituted Na^+ by K^+ ions, a re-established sodium gradient should help to control $[Ca^{2+}]_c$ despite excessive Ca^{2+} influx by promoting Ca^{2+} efflux via Na^+, Ca^{2+}-exchange. He added phosphate and creatine, pyruvate, etc. to facilitate generation of ATP. At this stage, I left for a sabbatical to Leuven. When I came back, Udo proudly demonstrated pre-incubated myocytes that were strong enough to withstand the exposure to normal $[Ca^{2+}]_o$. With the idea that the pre-incubation medium provided the myocytes with force for the successful fight against the Ca^{2+} paradox, we called the medium "Kraftbrühe" (power soup) or KB-medium. Although others have compared the KB-medium with witchcraft, I thought it was a discovery based on scientific ideas. Anyway, using cells from KB-medium we were the first to record Ca^{2+} currents (I_{Ca}) from isolated Ca^{2+} tolerant ventricular myocytes. Since Nature was not keen to publish "just" methodology, we combined our story with electron micrographs showing that the cells were naked in regard to the glycocalix [13]. Since the myocytes were "normal" in terms of the Ca^{2+} currents, we could discard the function of the glycocalix as a Ca^{2+} permeability barrier. The successful voltage-clamp experiments with isolated ventricular myocytes attracted visitors from all over the world, these collaborations being reflected in a variety of publications [14,15–20].

3.4. Ca^{2+} currents of isolated ventricular myocytes

In the first publication, we had voltage clamped the isolated myocyte with a single microelectrode. Later, we used two electrodes more carefully, current injection was separated from the electrode measuring the membrane potential. The two electrode clamp showed that I_{Ca} was 10 times faster and larger than described in multicellular trabeculae. We could demonstrate that the resistance in series with the cell membranes (single electrode or cell-to-cell resistance of the trabeculum) limit the charging of the membrane capacity. Since the current was no longer slow but relatively fast, we suggested to call it no longer "slow inward current" but "Ca^{2+} current", surprisingly, the cardiac electrophysiology community needed approximately 6 years to accept this suggestion [21,22].

3.5. Excitation–contraction coupling

Udo had left physiology for his residency, and I concentrated on the question whether the fast and large I_{Ca} could activate cardiac contractions without the necessity of SR Ca^{2+} release. For this, isotonic shortening of the isolated myocytes was measured by a video technique. I observed that the post-rest staircase in amplitude and rate of contraction occurred at constant Ca^{2+} influx per pulse. The result was incompatible with a direct activation of the myofilaments by Ca^{2+} from I_{Ca}. Instead, it supported the classical idea that most Ca^{2+} ions, entering with I_{Ca}, would be taken up by the SR, and that the filled SR could release more Ca^{2+} for the following contractions [23]. The studies

on isolated myocytes also suggested that Ca^{2+} currents and contractions could follow a different voltage dependence. When we stimulated the cells with action potentials at 1 Hz and interposed the clamp pulses, and found instead of the bell- but an S-shaped voltage dependence. At +100 mV we observed a twitch as large and fast as at 0 mV. Since Ca^{2+} influx should be negligible, we suggested that Ca^{2+} can be released from the SR without Ca^{2+} influx [24]. This speculation on depolarisation-induced Ca^{2+} release in ventricular myocytes is not yet resolved.

3.6. My love

I met Maria Fiora Gallitelli in October 1981 during the meeting organized in honour of Wilhelm Hasselbach. I had talked on I_{Ca} in relation to the shortening of the isolated myocytes. She gave a talk on electron probe microanalysis (EPMA) to show how the staircase changes the calcium load in intracellular compartments of cardiac trabeculae. Her talk let me dream: I would be a Ca^{2+} ion, would swim with I_{Ca} through the channels, and would finally disappear via Hasselbach's SERCA in the SR where Pitti could find me by EPMA. I dreamt I could quantify Ca^{2+} movements not only globally as "pA per cell" but would be able to distribute this flux into components between intracellular compartments. After her talk, I was addressing her with the "demand" (she remembers this way) "we should put ourselves together". Presumably, I was trying to say that EPMA of calcium concentrations in ventricular myocytes with defined Ca^{2+} influx would yield new important information. What originally began as a scientific collaboration turned into the great love of my life. I wonder whether we would have had the trust, the patience, and the success without this love. M.F. Gallitelli introduced herself as Pitti, the little, an Italian nickname given to her by her older sister. In 1998, we married in our late fifties, and we were happy that all four adult children (and one grandson) joined us in the wedding ceremony. We lived together. Even the genes did not meet in any of these children, we were most happy if children and grandchildren visit us in our house.

Pitti was working in Tübingen where she had the electron microscope equipped with EPMA. Pitti had the hands able to cryosect the specimen for later analysis and the brain to keep me on track. The first step, shock-freezing, was the most difficult one: since the specimen is analysed off line, the diffusional dissipation of the ion gradients should be avoided by rapidly freezing the cell to −195 °C at a rate where formation of ice crystals is negligible. For the trabeculae, Pitti worked with special forceps that were put into liquid propane. How could we adapt this first step? Initially, air pressure "shot" a microelectrode with the cell through on its tip through a hole in the chamber to a silver-plate cooled by liquid nitrogen. However, we found only pieces of glass and never the cell. In the following 3 years, Pitti developed a new tool, the "holder for shock freezing". The holder was manufactured from silver by hand. Pitti etched a small hole into it and closed the hole with a thin transparent film. For this experiment, I transported the cell on top of the film by means of the patch electrode. Through the film we could monitor the cell contractions with a video system. The holder served as a reference electrode for electrophysiology. For shock-freezing, the microscope was replaced by a beaker of liquid propane (−190 °C) that was shot upwards to freeze the holder with the cell and the electrode. That is, instead of shooting cell and electrode, both of them were kept still and all the other equipment was moved. Since the holder worked as reference electrode also outside the experimental chamber the currents could be measured until the contact with the coolant.

When we had performed sufficient experiments in Cologne, Pitti transported approximately six holders in liquid nitrogen to the University of Tübingen. She was able to mount the two legs of the holder into her cryomicrotome, where she was able to section silver, bathing solution, cell and electrode glass by a diamond. This cryocutting is really a work of art (and it is boring and very cold). Our happiness to see and to analyse these sections in the electron microscope was overwhelming. We could indeed quantify the fraction of Ca^{2+} current that fills the SR compartment (after a rest period). The results were difficult to publish. First of all, EPMA measures the concentration of total calcium which is complementary but different from the ionised $[Ca^{2+}]_c$. The comparison between changes of free $[Ca^{2+}]_c$ measured with fluorescent indicators with those of total calcium concentration obtained by EPMA indicated a Ca^{2+} buffer capacity in the mM range [25,26]. Many people did not believe such a high number because the necessary ligands were not known, e.g. the Ca^{2+} binding by actin was only published years later. Her results showing beat to beat changes of mitochondrial Ca [27] differed from those of other influential groups [28,29]. However, it is gratifying to note that, years later, her work has been supported by studies using entirely different techniques [30].

4. The golden times in Cologne (1985–1995)

In 1985, I obtained a full professorship in the Department of "Vegetative Physiology" at the University of Cologne. The department provided me with two scientific positions, one technician and a other little budget. Most importantly, it had the most wonderful machine shop. Many of the achievements would not have occurred without the help of these people, especially the development of shock-freezing of myocytes for EPMA. I lived in a one-room apartment close to the Institute, the walking distance being not longer than 7 min. Thus, I spent my life mostly in the nice rooms of the university and used the little apartment just for sleeping. This type of living was thought to be temporary, however, the family never moved from Homburg to Cologne, I finally got divorced.

I used a sabbatical to visit Ed Lakatta in Baltimore where I learned $[Ca^{2+}]_c$ measurements with the fluorescent indicator Indo-1. Together with Talo I was measuring Ca^{2+} signals in voltage-clamped rat ventricular cells. After some struggle with technical problems, we could find two components in the Ca^{2+} signal [31]. In contrast to the Purkinje fibre, the initial rapid component was due to SR Ca^{2+} release and the later slow component to Ca^{2+} influx.

Udo Klöckner followed to Cologne after a 1 year delay, and we could finished the work on isolated smooth muscle cells that had been started in Homburg. In the eighties, voltage-clamp of smooth muscle strips were usually clamped by the double sucrose gap, and the recorded currents were contaminated by artefacts typical for multicellular preparations. Dissociation of smooth muscle tissue into single myocytes should offer a more suitable preparation for voltage-clamp. Thereby, we hoped to enter a scientific field that was largely unexploited at these days. The field of smooth muscle physiology attracted us also because of new ideas such as receptor operated channels [32], pharmaco-mechanical coupling [33], and because of its importance for vascular physiology. We started with cells from the urinary bladder because this tissue was big and because the cells were large. Again, the L-type Ca^{2+} current was big and fast [34]. We attributed the pacemaking of the isolated urinary bladder myocytes to the deactivation of a Ca^{2+} activated big K^+ channel, superimposed on different small inward currents [35]. One more preparation had been taken out of the prejudice which declared that smooth muscle was too complex for voltage-clamp analysis. In Cologne and with a new preparation, we escaped from Trautwein's demand to stay away from single channel analysis. Udo analysed the L-type Ca^{2+} channel activity [36] as it is modulated by protons [37,38]. In addition, we analysed the Ca^{2+} dependency of the 200 pS K^+ channel that becomes active only at $[Ca^{2+}]_c$ higher than 1 μM. Since our $[Ca^{2+}]_c$ measurements reported values below 1 μM, we speculated that some narrow spaces underneath the sarcolemma should contain Ca^{2+} at concentrations much higher than global $[Ca^{2+}]_c$ in the cytosol [39]. With the idea of subsarcolemmal Ca^{2+} and Na^+ accumulation we introduced the concept of cytosolic ion heterogeneity, a concept I am still following up at present [40,41].

During the Cologne period, there were many guests in the lab, and I can only mention just some of them. With Neal Sheperd and Matti Vornanen, we succeeded to measure the force generated by the voltage-clamped ventricular myocyte [42]. Elisabetta Cerbai came as an undergraduate from Florence, Italy. Rapidly, she picked up all the secrets, and she finished with a Science paper on the interaction of G-protein subunits with the muscarinic K^+ channel [43]. Ryuji Inoue was sent from Kyushu to Cologne, together with his family. Despite being trained by the German Humboldt foundation, he had a difficult time to understand that the German rough answers and the many "nos" were not meant to hurt him. During his Cologne time, Riuji completed three papers on the mACh operated current in isolated ileal smooth muscle cells that is modulated by hyperpolarization and by Ca^{2+} ions via the Ca^{2+} channel current I_{Ca} [44,45]. The finding that mACh-activation could be blocked by pertussis toxin, and induced by GTPγS in the absence of the agonist led us to suggest that the muscarinic receptor is coupled to non-selective cation channels via a G-protein [46].

During a conference at the Ukrainian Academy, the Bogomoletz Institute of Physiolgy, I met Vladimir Ganitkevich. Vlado was fighting for the idea that STOCs (Ca^{2+}-activated K^+ currents) were a normal phenomenon whilst I called them a phenomenon of SR Ca^{2+} overload. Presumably, we met somewhere in between and became friends [47]. I invited Vlado to my lab in Cologne with the blessing of his Boss, Michael Shuba. He arrived after a long train ride on an early October morning, and I was late at the station to pick him up. Vlado had many creative ideas about what to do in Cologne, and soon he incorporated our Indo1-Ca^{2+}-set up into these ideas. His experiments produced a lot of new data. I did not have to do much more than to discuss ideas and data and to write the manuscripts. It was a fruitful time for both of us [41,48,49].

After a year delay, Vlado's wife and his two sons followed to Cologne. After 3 years, Platon Kostyuk recalled Vlado back to Kiev, and Vlado did not go. I remember my visit to Kiev in 1988 when we talked about Vlado's idea to stay in Cologne. I was wondering whether the man in front of me was the same Platon Kostyuk I had met on the Western side of the iron curtain, and I remembered my own brain-split in the past. Essentially, Kostyuk argued that western society would steal the brains from the Soviet Union. I admit that there is some truth in this. Kostyuk, on the other hand, did not accept the idea that a young scientist, Vlado Ganitkevich, could realise his own ideas, wills and decisions.

5. Halle 1995

The books of German history tell us about the "soft revolution" in 1989 that finished the former GDR and finally led to unification. When this happened, I was in Cologne and immediately bought a TV. The wall opened for me in December 1989, and I could travel to the East to see my mother in law and my friends for the first time since 1972. During 1990, university life in the former GDR was re-organized and some careers abruptly started or ended. It was obvious that the re-painted communists were still in power and that they were using this power for controlling the re-organization. My friend Walter Jahn in Leipzig and I addressed the problem with a public letter in one of leading German weekly journals. Our letter had helped the Medical faculty in Leipzig. However, it also had the consequence that Walter Jahn lost his position as a university professor of stochastics. Later, between 1990 and 1992, scientists without a known link to SED or Stasi organized a round table and decided who had to be fired from university and

who could stay. Usually, this evaluation asked: did he or she harm other people for political reasons? Unavoidably, the "objective" arguments were superimposed on personal likes and dislikes. I was more than happy that these "evaluations" had ended when I moved to the East of Germany.

In 1994, I was offered the chair of the department of Physiology at the Martin-Luther-Universität Halle-Wittenberg, and I accepted in February 1995. Pitti followed me in September 1995. The department was named "Julius-Bernstein-Institut" after Julius Bernstein who had measured membrane resting and action potentials in nerve and muscle in 1868 and founded the "membrane theory" in terms of diffusion potentials in 1912. The department had been chaired until 1992 by Leo Zett who had obtained the chair in 1973 as a member of the SED. Leo Zett had worked as dean of the medical Faculty in Halle during 1981–1989, and for this he was fired in 1992. I heard only reasonable or good comments about his behaviour.

My plan of moving Udo and Vlado to Halle did not work. Partially, this was because the city of Halle was dirty city and non-attractive, in contrast to Cologne. Also, I could not offer them jobs better than those in Cologne. Presumably, they also liked to demonstrate that they were the real heroes of the Cologne group. Thus, Pitti and I started in Halle alone.

I had several reasons for "going back to East". Firstly, in Germany, chairmanship is linked to a lot of prestige that I did not like to miss. Secondly, a lot of money was given to East German universities to re-build their infrastructure. Thus, I thought I could establish a group of good scientists that would work along the concept "physiology of cell compartments" in cardiovascular myocytes. Finally, I felt "obliged" to pay back the good things I had received in Leipzig between 1960 and 1972. Most of these dreams I did not realise, and often I had regretted my move. As a chairman I spent a lot of time with administrative things for which I neither had the talent nor the education. By law, we teach 8 h a week, a duty that easily turns into 20 h a week, if one prepares the lectures and seminars, and if one gives the students a chance to repeat their examinations. The departmental funding has been reduced every year since 1996; whilst this reduction was acceptable in the beginning, it is now corroding the basis of scientific life in Halle and in many other German universities.

5.1. Collaborations

Despite some trouble, there were many things to enjoy. Finally, I could work with Pitti in the same place. We bought a small house at the city border, and we filled our home with peace and happiness. In 1994, we had started a collaboration with *Fred Fay* applying his imaging techniques to our concept of cytosolic ion heterogeneity. In 1996, I got an award supporting this collaboration. Thus, Pitti and I travelled to Worcester (MA), and Fred came to Halle. During his stay in March 1996, Fred presented the work with a brilliant talk at a meeting in Halle [50]. Afterwards, we went to the lab and Fred called his office from my desk. When he did not come back after a while, I entered my office and found Fred dead on my chair, the telephone receiver in his hands. All attempts at resuscitation failed. Fred's wife Madeleine and little daughter Isabelle came over 3 months later, to see the cemetery where husband and father had been cremated. We are thankful that the friendship between the families survived the terrible event. Fred's death and its circumstances remained my nightmare for at least 2 years.

Andrej Kamkin and Irina Kiseleva from Moscow joined the group in 1999. Andrej introduced the idea of the "mechano-electrical feedback" to us. He had studied the effect of stretch on the membrane potential of multicellular atrial trabeculae [51]. He was now eager to learn more about the cellular mechanisms of these stretch-induced depolarisations. He arrived with the idea to repeat experiments in ventricular myocytes that Marie-Cecile Wellner had done in Cologne with smooth muscle cells and that had told us an understanding how currents through stretch activated non-selective cation channels determine the spontaneous activity [52,53]. In 1999, we had sufficient money to buy equipment for stretching individual isolated ventricular myocytes. Andrej could measure the stretch-activation of Gd^{3+}-sensitive inward currents that generated membrane depolarisations, after potentials and eventually cellular arrhythmias [54–56]. Andrej never gave up his job as a chairman in Moscow, however, he supplied our department with good young Russian scientists. The mechano-electrical feedback finally ended in a Collaborative Research Center (Sonderforschungsbereich or SFB in German) entitled "mechanical modulation of the phenotype of cardiovascular cells" where departments from Goettingen, Hannover and Halle work together and to which our group is contributing most of the electrophysiology [57–59].

The severe financial problems of the local state Sachsen-Anhalt and of our University started the discussion that the Medical faculty in Halle should be closed. In this situation, the existence or non-existence of a SFBs at the Medical faculty was an important survival criterion. Thus, we struggled to find a cardiovascular SFB in our faculty. The SFB was planned for 1999, but it was only achieved in 2002. Because of limited man power, interactions between Cardiology, Cardiac Surgery, Pharmacology, Physiology and Biochemistry were joined into the "Collaborative Research Center". In addition, reviewers and government needed a convincing theme. Since our society is ageing and the elderly suffer and die from cardiovascular diseases, we convinced referees and politicians with the fashionable title "Heart failure in the elderly: cellular mechanisms and possible therapies". In the last years, we could put forward experiments describing the pecularities of the cardiomyocytes isolated from senescent mice and rats [60,61]. Some friends disliked my use of political arguments to find money resources, considering this as a type of corruption. Of course I wish science should be "free", however, I also understand that society expects "something useful to come

out from all this money". Anyway, when the SFB was positively reviewed and started in 2002, it ended the present discussion about the closure of our Medical Faculty.

6. Future for physiology

The word "fashion" sounds dirty if it is used in context with science. I think, science is always concentrated in fashionable fields. In part, the fashion depends on the power of new methods, as I had experienced with isolated cells, patch clamp and single channel analysis. In addition, the fashion is governed by money, sometimes in accord with the demands of the society. We experience now an overwhelming influence of Molecular Biology and Neurosciences. I think that this also is a fashion and that this fashion is partially connected to the hope then one could apply it to medicine to make big money. I experience that Molecular Biology and Neurosciences dominate by a positive feedback, highly cited journals prefer to publish fashionable results and send to an outsider the standard letter that declares the submitted manuscript were "not interesting enough for the wide audience". The positive feedback generates a lobby that is involved in the decision on grant proposals, fashionable topics go through easier whilst an application not including the fashion has problems to become funded.

Instead of fashion I could have used the word "modern science" and ask whether the scientific community considers physiology to be "non-fashionable" or "outdated"? As a young man, I became a physiologist with the desire to better understand the function of the healthy body. Close to retirement, this desire is still vivid, partly because scientific progress always uncovers new problems. In addition, there is a new task: the successful genome project has provided a lot of information, and physiology is asked to link this information to function. Physiology is integrating the function of small units, such as proteins, compartments, cells, and it studies how these units build up the function of a cell, a tissue, an organ and an organism. For me physiology will always be a fashionable science, a science that not only dissects objects and functions to smaller and smaller identities but also integrates them in such a way that we better understand our living body better.

References

[1] B. Merrem, G. Küchler, G. Isenberg, Zur ableitung bioelektrischer potentiale von einzelnen skelettmuskelfasern mit der saccharosetrennwandmethode. I. Einfluß der versuchsbedingungen auf das präparat, Acta Biol. Med. Germany 21 (1968) 51–65.

[2] G. Isenberg, Voltage-clamp experimente an isolierten skelettmuskelfasern unter kombination von mikroelektroden- und saccharosetrennwandtechnik, Acta Biol. Med. Germany 26 (1971) 311–321.

[3] P.G. Kostyuk, O.A. Krishtal, V.I. Pidoplichko, Effect of internal fluoride and phosphate on membrane currents during intracellular dialysis of nerve cells, Nature 257 (1975) 691–693.

[4] K.A. Deck, W. Trautwein, Ionic currents in cardiac excitation, Pflugers Arch. 280 (1964) 63–80.

[5] G. Isenberg, W. Trautwein, The effect of dihydro-ouabain and lithium ions on the outward current in cardiac Purkinje fibres. Evidence for electrogenicity of active transport, Pflügers Arch. Eur. J. Physiol. 350 (1974) 41–54.

[6] J. Vereecke, G. Isenberg, E. Carmeliet, K efflux through inward rectifying K channels in voltage clamped Purkinje fibres, Pflügers Arch. Eur. J. Physiol. 384 (1980) 207–217.

[7] N. Akaike, A.M. Brown, G. Dahl, et al., Voltage-dependent activation of potassium current in Helix neurones by endogenous cellular calcium, J. Physiol. 334 (1983) 309–324.

[8] G. Dahl, G. Isenberg, Decoupling of heart muscle cells: correlation with increased cytoplasmic calcium activity and with nexus ultrastructure, J. Membr. Biol. 53 (1980) 63–75.

[9] G. Wier, G. Isenberg, Intracellular $[Ca^{2+}]_i$-transients in voltage clamped cardiac Purkinje fibres, Pflügers Arch. Eur. J. Physiol. 392 (1982) 284–290.

[10] W. Trautwein, T.F. McDonald, O. Tripathi, Calcium conductance and tension in mammalian ventricular muscle, Pflügers Arch. 354 (1975) 55–74.

[11] C. Baumgarten, G. Isenberg, Depletion and accumulation of potassium in the extracellular clefts of cardiac Purkinje fibres during voltage clamp hyperpolarization and depolarization, Pflügers Arch. Eur. J. Physiol. 368 (1977) 19–31.

[12] T. Powell, D.A. Terrar, V.W. Twist, Electrical properties of individual cells isolated from adult rat ventricular myocardium, J. Physiol. 302 (1980) 131–153.

[13] G. Isenberg, U. Klöckner, Glycocalix is not required for slow inward calcium current in isolated rat heart myocytes, Nature 284 (1980) 358–360.

[14] G. Van der Heyden, J. Vereecke, G. Isenberg, G. Callewärt, E. Carmeliet, Metabolic inhibition and the cardiac ventricular action potential: voltage clamp analysis using single cells, Arch. Int. Pharm. Ther. 263 (1983) 331–332.

[15] G. Isenberg, U. Ravens, The effects of the *Anemonia sulcata* toxin (ATX II) on membrane currents of isolated mammalian myocytes, J. Physiol. (Lond.) 357 (1984) 127–149.

[16] L. Belardinelli, G. Isenberg, Isolated atrial myocytes: adenosine and acetylcholine increase potassium conductance, Am. J. Physiol. 244 (1983) H734–H737.

[17] G. Isenberg, L. Belardinelli, Ionic basis for the antagonism between adenosine and isoproterenol in isolated guinea pig ventricular myocytes, Circ. Res. 55 (1984) 309–326.

[18] M. Horackova, A. Beresewicz, G. Isenberg, Effect of reduced Na gradient on electrical activity in isolated bovine and feline ventricular myocytes, Can. J. Physiol. Pharmacol. 66 (1988) 222–232.

[19] L.E. Moore, A. Schmid, G. Isenberg, Linear electrical properties of isolated cardiac cells, J. Membr. Biol. 81 (1984) 29–36.

[20] G. Isenberg, J. Tamargo, Effect of imipramine on calcium and potassium currents in isolated bovine ventricular myocytes, Eur. J. Pharmacol. 108 (1985) 121–131.

[21] G. Isenberg, U. Klöckner, Calcium tolerant ventricular myocytes prepared by preincubation in a "KB Medium", Pflügers Arch. 395 (1982) 6–18.

[22] G. Isenberg, U. Klöckner, Calcium currents of isolated bovine ventricular myocytes are fast and of large amplitude, Pflügers Arch. Eur. J Physiol. 395 (1982) 30–41.

[23] G. Isenberg, Ca entry and contraction as studied in isolated bovine ventricular myocytes, Z. Naturforsch. 37c (1982) 502–512.

[24] G. Isenberg, A. Beresewicz, D. Mascher, F. Valenzuela, The two components in the shortening of unloaded ventricular myocytes: their voltage dependence, Basic Res. Cardiol. 80 (Suppl. 1) (1985) 117–122.

[25] G. Isenberg, M.F. Wendt-Gallitelli, Binding of calcium to myoplasmic buffers contributes to the frequency-dependent inotropy in heart ventricular cells, Basic Res. Cardiol. 87 (1992) 411–417.

[26] M.F. Wendt-Gallitelli, G. Isenberg, Total and free myoplasmic calcium during a contraction cycle: X-ray microanalysis in guinea-pig ventricular myocytes, J. Physiol. (Lond.) 435 (1991) 349–372.
[27] M.F. Wendt-Gallitelli, G. Isenberg, Time course of myofibrillar calcium during the cardiac cycle. X-ray microprobe analysis of voltage-clamped guinea-pig ventricular cells, J. Physiol. (Lond.) 435 (1991) 349–372.
[28] A.P. Somlyo, A.V. Somlyo, Electron probe analysis of calcium content and movements in sarcoplasmic reticulum, endoplasmic reticulum, mitochondria, and cytoplasm, J. Cardiovasc. Pharm. 8 (2003) s42–s47.
[29] R.Y. Ho, D.S. Fan, A.V. Somlyo, A.P. Somlyo, Calcium content of peripheral and central mitochondria in the guinea pig myocardium: electron probe analysis, Cell Calcium 33 (2003) 247–256.
[30] G.A. Rutter, P. Burnett, R. Rizzuto, et al., Subcellular imaging of intra-mitochondrial Ca^{2+} with recombinant targeted aequorin: significance for the regulation of pyruvate dehydrogenaseactivity, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 5489–5494.
[31] A. Talo, M.D. Stern, H.A. Spurgeon, G. Isenberg, E.G. Lakatta, Sustained subthreshold-for-twitch depolarization in rat single ventricular myocytes causes sustained calcium channel activation and sarcoplasmic reticulum calcium release, J. Gen. Physiol. 96 (1990) 1085–1103.
[32] C.D. Benham, ATP-activated channels gate calcium entry in single smooth muscle cells dissociated from rabbit ear artery, J. Physiol. (Lond.) 419 (1989) 689–701.
[33] A.P. Somlyo, A.V. Somlyo, Signal transduction and regulation in smooth muscle, Nature 372 (1994) 231–236.
[34] U. Klöckner, G. Isenberg, Calcium currents of cesium loaded isolated smooth muscle cells (urinary bladder of the guinea-pig), Pflügers Arch. 405 (1985) 340–348.
[35] U. Klöckner, G. Isenberg, Action potentials and net membrane currents of isolated smooth muscle cells (urinary bladder of the guinea-pig), Pflügers Arch. 405 (1985) 329–339.
[36] U. Klöckner, A. Schiefer, G. Isenberg, L-type Ca-channels: similar Q_{10} of Ca-, Ba- and Na-conductance points to the importance of ion-channel interaction, Pflügers Arch. 415 (1990) 638–641.
[37] U. Klöckner, G. Isenberg, Intracellular pH modulates the availability of vascular L-type Ca^{2+} channels, J. Gen. Physiol. 103 (1993) 647–663.
[38] U. Klöckner, G. Isenberg, Currents through single L-type Ca^{2+} channels studied at 2 mM $[Ca^{2+}]_o$ and 36 °C in myocytes from the urinary bladder of the guinea-pig, J. Physiol. (Lond.) 438 (1991) 228.
[39] U. Klöckner, G. Isenberg, ATP suppresses activity of Ca^{2+}-activated K^+ channels by Ca^{2+} chelation, Pflügers Arch. 420 (1992) 101–105.
[40] M.F. Wendt-Gallitelli, T. Voigt, G. Isenberg, Microheterogeneity of subsarcolemmal Na concentrations in guinea-pig ventricular cells. An X-ray microprobe study, J. Physiol. 472 (1993) 33–44.
[41] V.Y. Ganitkevich, G. Isenberg, Dissociation of subsarcolemmal from global cytosolic $[Ca^{2+}]$ in myocytes from guinea-pig coronary artery, J. Physiol. (Lond.) 490 (1996) 305–318.
[42] N. Shepherd, M. Vornanen, G. Isenberg, Force measurements from voltage-clamped guinea pig ventricular myocytes, Am. J. Physiol. 258 (1990) H452–H459.
[43] E. Cerbai, U. Klöckner, G. Isenberg, The α-subunit of the GTP binding protein activates muscarinic potassium channels of the atrium, Science 240 (1988) 1782–1783.
[44] R. Inoue, G. Isenberg, Effect of membrane potential on acetylcholine-induced inward current in guinea-pig ileum, J. Physiol. (Lond.) 424 (1990) 57–71.
[45] R. Inoue, G. Isenberg, Intracellular calcium ions modulate acetylcholine-induced inward current in guinea-pig ileum, J. Physiol. (Lond.) 424 (1990) 73–92.
[46] R. Inoue, G. Isenberg, Acetylcholine activates nonselective cation channels in guinea pig ileum through a G protein, Am. J. Physiol. Cell Physiol. 258 (1990) C1173–C1178.
[47] V. Ganitkevich, G. Isenberg, Isolated guinea pig coronary smooth muscle cells: acetylcholine induces hyperpolarization due to sarcoplasmic reticulum calcium release activating potassium channels, Circ. Res. 67 (1990) 525–528.
[48] V.Y. Ganitkevich, G. Isenberg, Efficacy of peak Ca^{2+} currents (I_{Ca}) as trigger of sarcoplasmic reticulum Ca^{2+} release in myocytes from the guinea-pig coronary artery, J. Physiol. (Lond.) 484 (1995) 287–306.
[49] V.Y. Ganitkevich, G. Isenberg, Membrane potential modulates inositol 1,4,5-trisphosphate-mediated Ca^{2+} transients in guinea-pig coronary myocytes, J. Physiol. (Lond.) 470 (1993) 35–44.
[50] G. Isenberg, E.F. Etter, M.F. Wendt-Gallitelli, et al., Intrasarcomere $[Ca^{2+}]$ gradients in ventricular myocytes revealed by high speed digital fura-2 imaging, PNAS 93 (1996) 5413–5418.
[51] A. Kamkin, I. Kiseleva, K.D. Wagner, et al., A possible role for atrial fibroblasts in postinfarction bradycardia, Am. J. Physiol. 282 (2002) H842–H849.
[52] M.C. Wellner, G. Isenberg, Properties of stretch activated channels in myocytes from the guinea-pig urinary bladder, J. Physiol. (Lond.) 466 (1993) 213–227.
[53] M.C. Wellner, G. Isenberg, cAMP accelerates the decay of stretch-activated inward currents in guinea-pig urinary bladder myocytes, J. Physiol. (Lond.) 482 (1995) 141–156.
[54] A. Kamkin, I. Kiseleva, G. Isenberg, Stretch-activated currents in ventricular myocytes: amplitude and arrhythmogenic effects increase with hypertrophy, Cardiovasc. Res. 48 (2000) 409–420.
[55] A. Kamkin, I. Kiseleva, G. Isenberg, Activation and inactivation of a non-selective cation conductance by local mechanical deformation of acutely isolated cardiac fibroblasts, Cardiovasc. Res. 95 (2003) 1.
[56] G. Isenberg, V. Kazanski, D. Kondratev, M.F. Gallitelli, I. Kiseleva, A. Kamkin, Differential effects of stretch and compression on membrane currents and $[Na^+]_c$ in ventricular myocytes, Prog. Biophys. Mol. Biol. 82 (2003) 43–56.
[57] A. Hipper, G. Isenberg, Cyclic mechanical strain decreases the DNA synthesis of vascular smooth muscle cells, Pflügers Arch. Eur. J. Physiol. 440 (2000) 19–27.
[58] U. Rueckschloss, G. Isenberg, Cytochalasin D reduces Ca^{2+} currents via cofilin-activated depolymerization of F-actin in guinea-pig cardiomyocytes, J. Physiol. (Lond.) 537 (2001) 363–370.
[59] S. Wagner, E. Picht, L. Maier, et al., Na^+/Ca^{2+} exchanger overexpression predisposes to reactive oxygen species-induced injury, Cardiovasc. Res. 60 (2003) 404–412.
[60] B. Husse, A. Sopart, G. Isenberg, Cyclical mechanical stretch induced apoptosis in myocytes from young rats but necrosis in myocytes form old rats, Am. J. Physiol. Heart Circ. Physiol. 285 (2003) H1521–H1527.
[61] G. Isenberg, J.U. Borschke, U. Rueckschloss, Ca^{2+} transients of cardiomyocyte from senescent mice peak late and decay slowly, Cell Calcium 34 (2003) 271–280.