Cesium Determination in Physiological Fluids and Tissues by Field Desorption Mass Spectrometry †

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Quantitative ultratrace analysis (10 nmol to 10 μmol/l) of cesium in biological samples such as human body fluids and animal tissues is performed without any prior purification or concentration steps. The normal level of cesium ions in heart cells was determined. After poisoning these cells with high concentrations of the alkali cation much higher levels were found inside the cells then had been suggested previously. It is demonstrated that field desorption mass spectrometry is a unique tool for the qualitative and quantitative investigations of metal cations in biological material.

Introduction

The physiological milieu of living cells mainly consists of alkali and alkaline earth ion solutions. The different concentrations of sodium-, potassium- and calcium-ions in the various compartments of a living organism as well as the transport of these ions through cell membranes provides the basis of all physiological processes and is therefore decisive for the function of these organisms.

In the body cesium ions occur in traces only and very recently investigations have been made to determine the concentration of these ions in body fluids [1]. An important toxic effect of higher cesium concentrations in body is the blockage of potassium currents through biological membranes. The study of these effects requires an analytical method which is able to detect cesium concentrations in very small samples (*e. g.* milligrams of tissue material) down to a range of ppb (1 ppb cesium = 1 μg cesium/l). In the following it will be demonstrated that Field Desorption (FD) – Mass Spectrometry (MS) is able to fulfill these demands because of its outstanding sensitivity for metal cations and its high specificity. In comparison to this method Atomic Absorption Spectrometry has a lower sensitivity for cesium [2, 3]. Comparable sensitivity in detecting cesium is only achieved with radiochemical analysis [4] and surface-ionization mass spectrometry [5, 6]. The suitability of FD-MS for the determination of alkali ions [7] and especially for cesium [1, 8] was already described. In the FD spectra of commercially available pesticides and dyestuffs [9] and of natural products [10] containing alkali salts the respective alkali cations appear with high intensity relative to the ions derived from the organic constituents of the sample. In particular this will happen when the emitter on which the sample is deposited is heated from room temperature to red heat [11], because metal cations generally desorb at higher emitter temperatures than the organic material. In this same treatment one can detect signals of stable complexes between organic molecules (M) and metal cations $[Ca]^+$ in form of $[M + Cat]^+$ ions [12–14]. Absolute cesium amounts of about 50 fg (10^{-15} g) per emitter are sufficient for qualitative and quantitative determinations [1].

In investigations of electrophysiological effects at cell membranes of the excitable system in the heart the pacemaker current has been attributed to a potassium outward current [15–17], the kinetics of which are controlled via beta receptors [18–21].

Isenberg [22] showed that a concentration of 1 mmol/l cesium in Tyrode solution as the outer milieu of cardiac Purkinje fibres – part of the excitable system of the heart – is sufficient to suppress the potassium outward current (pacemaker current) nearly completely. When the concentration of cesium exceeds 20 mmol/l all detectable potassium currents in cardiac Purkinje fibres are blocked. Since this effect was to be seen within one or two minutes it was believed that cesium ions are able to block the potassium channels in the membrane from the outside [22]. One possible explanation would be based upon the fact that cesium ions are bigger than potassium ions

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(diameter $[Cs]^+ = 1.77$ Å; $[K]^+ = 1.33$ Å [23]). Therefore, if they eventually invade the potassium channels, they should be hampered in their motion or even be immobilized because of their bigger size. If this should be the case, cesium ions would invade potassium channels whenever they reach cell membranes and would irreversibly block them. Besides, cesium ions should not be detectable inside the cells of the excitable system, since they are unable to penetrate the cell membranes.

From the standpoint of all classical microanalytical experiments of alkali ions in organic cell materials this hypothesis seemed to be correct. However, the only reason therefore was that a concentration of cesium which would be enough to block all potassium channels (about 100 nmol/l) was too small for such measurements. Calculations of the effective number of sodium channels were done by Moore, Narahashi and Shaw [24]. Considering the measurements of Mobley and Page [25] such a calculation for potassium channels in the membrane up to 20 channels/μm^2 seemed to be realistic. Thus, a cesium concentration of about 100 nmol/l should be sufficient to block every potassium channel with one cesium ion.

By means of FD-MS a cesium concentration of 135 nmol/l was found in human blood [1]. This measurement alone leads to some doubts about the hypothesis of blockage from the outside of the membrane described above, since all channels should be blocked under normal conditions. In order to check this hypothesis and because the method of FD-MS is even quantitatively able to determine cesium concentrations down to about 0.1 nmol/l, measurements by this technique were made with untreated Purkinje fibres and heart muscle from sheep as well as human saliva and urine. In addition, experiments with Purkinje fibres and heart muscle cells were done after treatment of the whole heart for 1.5 respectively 30 min with a Tyrode solution containing 1 mmol/l cesium.

Methods

a) Field desorption technique

The ion currents – produced by FD – were registrated in a single focussing mass spectrometer which was equipped with an home-built ion source. The FD emitter – at a potential of +8 kV – was located at a distance of 2 mm from the counter electrode (at −3.5 kV). Only the first lens of the ion source was used for focussing of the ion beam, all other deflection plates were at ground potential. This experimental setup simplifies the operation of the FD mass spectrometer considerably because it allows an easy, fast, and reproducible optimizing of the FD ion currents; this being particularly relevant for quantitation. With an entrance slit width of about 0.5 mm and an exit slit width of about 1.0 mm, a resolution of about 200 (10% valley definition) was achieved. The adjustment for the maximum cesium current could be done for every measurement within 2 min. The time for one experiment – including all measurements for a special calibration curve for every new sample – was about 1.5 h. The ions were detected by a simple secondary electron multiplier which operated at −3 kV. Field anodes employed were 10 μm tungsten wires activated at high temperature [7]. This process results in the formation of carbon microneedles. The average length of the microneedles was about 30 μm. A linearly programmed emitter heating current was used for the desorption of the samples. The determination of the cesium peak (at m/e 133) was performed at emitter heating currents between 0 mA and 80 mA corresponding to 30 °C and 1,500 °C, respectively. The programmer raised the emitter heating current at the rate of 0.04 mA per second. The desorption was measured by registration of the cesium ions at a constant magnetic field (single ion monitoring). In the calibration curves the area under the desorption profiles was plotted against the amount of cesium on the emitter.

Only emitters were used which showed a similar sensitivity for cesium currents. A calibration curve for the range of 10 nmol/l up to 1 mmol/l cesium was established for every emitter. These calibration curves were identical within the normal error range (approximately ±10%). Before determining a calibration curve – beginning with the lowest concentration – the position of the emitter inside the ion source was optimized with acetone (at m/e 58) at 0 mA emitter heating current by two micromanipulators. Stock solutions were made with CsCl (Suprapur, Merck AG, Darmstadt) with doubly-destilled water.

For every calibration curve 0.5 μl, 1.0 μl and 2.0 μl solution were deposited on the emitters by a modified syringe technique [26]. The curves for

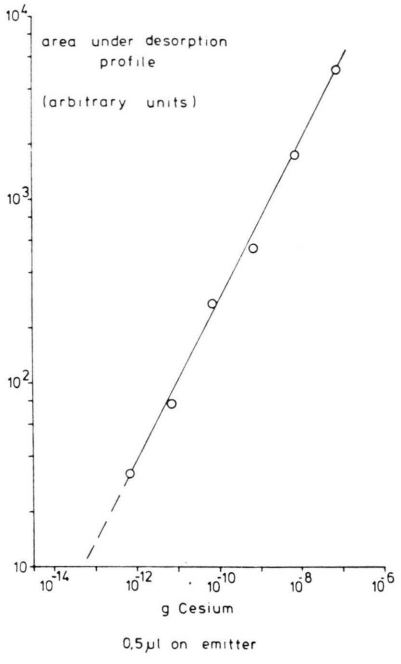

Fig. 1. Calibration curve for cesium ion current measurements. The area under the desorption profiles is plotted against the amount of cesium on the emitter. Ordinate: arbitrary units; abscissa: g cesium on the emitter.

0.5 μl solution (see Fig. 1) showed less deviations than the others. Larger amounts of samples on the emitter extrapolated to a better limit of detection but also to larger variations in the single measurements. Therefore all further experiments were performed using 0.5 μl of solution. For measurements of metal cations with a minimum of two stable isotopes it is not necessary to take up such calibration curves. If solutions are available in which one isotope is much more concentrated than its natural distribution, quantitative measurements can be done *via* the isotope dilution method. This procedure accelerates the whole measurement [27] and produces quantitative results of higher accuracy [28 – 30].

To test the possibilities of the FD-MS method the normal cesium concentrations in some body fluids and tissues were measured. Samples of human urine were taken two to four times in the same day, combined, and then analysed. The same procedure was followed for saliva. These solutions were deposited on the emitter by the syringe technique without any pretreatment (see Table I).

b) Preparations

Purkinje fibres and heart muscle cells for these experiments were prepared from sheep hearts which came from a slaughterhouse near Bonn (Kind, Grevenbroich). They were transported in Tyrode solution under carbogen (95% O_2 and 5% CO_2) at a temperature of about 10 °C. The whole hearts were exposed to a Tyrode solution containing 1mmol/l cesium for either 1.5 min or 30 min. Since Purkinje fibres are situated upon the surface of the heart muscles in the cavity of the heart ventricles, an adequate contamination of the fibres by the cesium solution was guaranteed. After this procedure the hearts were washed out for 30 min with normal Tyrode solution. Then the Purkinje fibres and heart muscle cells were separated from the heart and the Purkinje fibres were additionally washed out for 20 min with 2 l Tyrode solution in a special apparatus to make sure that no cesium would be left in the outercellular spaces of the fibres. The samples were carefully homogenised, Purkinje fibres with a special amount of doubly-distilled water. Therefore, the cesium contents of Purkinje fibres were obtained by multiplying the observed cesium amounts by an appropriate dilution factor.

Results and Discussion

Fig. 2 shows two typical desorption profiles for cesium ions measured by the secondary electron multiplier and using the same emitter. The hump in the profile from Purkinje fibres was in all experiments more or less pronounced. One could interprete this peak form by assuming that the organic material on the emitter is pyrolysed at lower temperatures and therefore some interference will occur at m/e 133. After all organic material is reduced to ashes the normal cesium current peak occurs as in simple salt solutions. The somewhat lower cesium concentrations found in earlier experiments [1] could have been the result of initially heating the emitter without the electric field in order to pyrolyze the organic material. In the measurements presented here the emitter was not heated before recording the cesium ion current. Table I shows examples of these measurements. For each sample the calibration curve was redetermined.

It is interesting that human urine contains concentrations of cesium as small as doubly-distilled

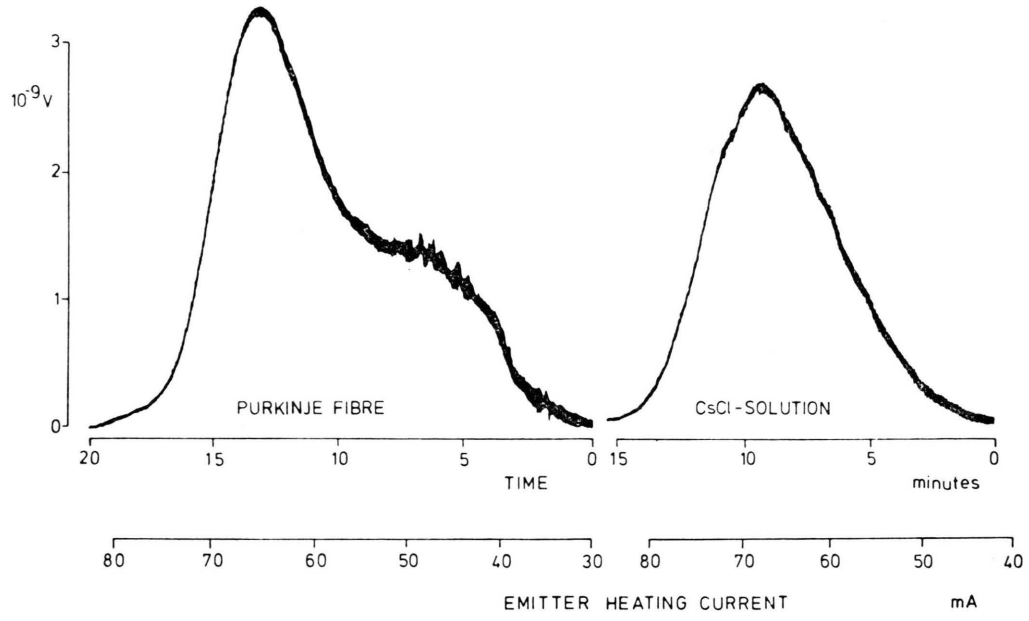

Fig. 2. Typical desorption profiles of cesium ions measured by a secondary electron multiplier. The left one is taken from a measurement of Purkinje fibres, the right one from a pure cesium chloride solution in doubly-distilled water. Both measurements were made with the same emitter.

water. At the same time there is much more cesium in human saliva – possibly the result of breathing (aerosols), diet or smoking. Membrane and filter systems as they are found in kidneys seem to be able to select cesium ions. Thus, the elimination of cesium from the body must go a pathway other than through the kidneys and urine.

Cesium concentrations in untreated Purkinje fibres and heart muscle were measured in a range of 880 nmol/l up to 1070 nmol/l.

According to the calculations on channel numbers this is sufficient to block all potassium channels in the membranes. If cesium ions are able to block the potassium outward current in the excitable system of the heart at all, they do not do so by plugging the channels from the outside of the membrane. After application of Tyrode solution containing 1 mmol/l cesium for only 1.5 min and a prolonged wash out period cesium concentrations in Purkinje fibres and heart muscle tissues increased to values between 14 μmol/l and 19 μmol/l. When a duration of application of 30 min was used, the concentrations rose up to 42 μmol/l to 71 μmol/l (see Fig. 3). These relatively high concentrations measured by FD-MS can also be detected by other methods with adequate accuracy, although the FD-MS method has the advantage that tissues need no pretreatment. Parallel measurements of the same preparations by atomic absorption and emission spectroscopy showed no significant deviations from our results.

Table I. Determination of cesium ions in body fluids and tissues by field desorption mass spectrometry without any pretreatment.

	Concentration of cesium [nmol/l]
Doubly-distilled water	9.5
Tyrode solution	80
Human urine	9.8
Human saliva	7,100
Heart muscle tissues	880
Purkinje fibres	1,100

Hence, Purkinje fibres take up so much cesium after an application of a cesium solution described above that the intracellular cesium concentration is still about 100 times higher after wash out than under normal conditions. The speed of this take up is indicated by the measurement after 1.5 min of treatment with cesium. That cesium ions are transported very quickly through the cell membranes into the cells is not astonishing because the concentration gradient from the outside to the in-

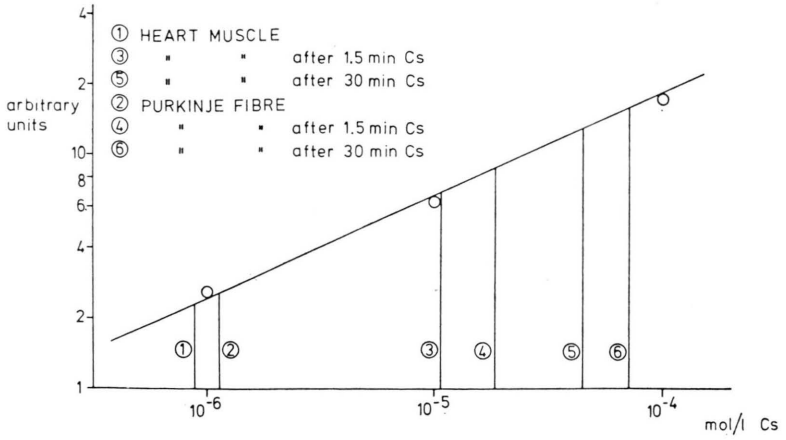

Fig. 3. Measurements of Purkinje fibres and heart muscle tissues at a typical calibration curve. The preparations were measured under normal conditions, after 1.5 min and after 30 min of application of a 1 mmol/l cesium solution and a prolonged period of wash out. Ordinate: arbitrary units, abscissa: concentration of cesium (mol/l).

side of the membrane is rather high (1 mmol/l to 1 μmol/l). If cesium ions are transported in a solvated form into the cells, one has to consider that their radius is somewhat smaller than that of solvated potassium ions. An exchange of cesium ions for potassium in leakage or pump currents would be possible. For the pacemaker outward current, however, potassium ions have to pass in an unsolvated form through special potassium channels. But for the unsolvated ions cesium is larger than potassium. One can imagine two possible mechanisms by which cesium can prevent the potassium transport: Firstly, blockage of potassium channels from the inner surface of the membrane or secondly, a very slow transport of cesium ions through potassium channels. If at the inner surface of the membrane the potassium ion concentration is about 0.15 mol/l and the cesium ion concentration is about 15 μmol/l and both ions want to pass through the same channel system, cesium ions would need 100,000 times longer than potassium ions to migrate through these channels. This is valid, if the pacemaker current is reduced to 10% of its normal value.

The FD-MS method allows traces and ultratraces of metal cations in body tissues and fluids to be measured. One can expect that using this technique it will be possible to do investigations which will help to explain experimentally observed electrophysiological effects. In particular a combination of FD-MS with electrophysiological voltage clamp experiments for measurements of ion currents in biological membranes seems to be reasonable and promising.

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