Improvement of the Accuracy by the Measurement of the Electrical Cell Membrane Parameters

V. ROHLÍČEK, F. RECH

Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic

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Summary
The electrical parameters of the cell membrane are mostly estimated employing ac methods. The measurement is based on the analysis of the current(s) flowing through an access resistance and the membrane. A current/potential transducer is used at the input of the device. The parameters of this transducer, especially its feedback capacity, degrades the accuracy of the measurement and hence diminishes the suppression of mutual influences of the individual parameters. The paper suggests a possible software correction and is supplemented by remarks for practical application.

Key words
Patch clamp technique • Electrical parameters of the cell membrane • Membrane capacity • Membrane conductance • Access resistance • Dual frequency method • Exocytosis

Introduction
The first estimation of membrane capacity was described by (Cole 1935), however, his technique did not allow the measurement of small changes of cell membrane capacity. The use of intracellular microelectrodes for electrical measurement was published by Jaffe et al. (1978). Just the introduction of the whole cell patch clamp technique together with synchronous detection (Neher and Marty 1982) attained the necessary resolution power for the monitoring of small membrane capacity changes, for instance single events by exocytosis. The detailed analysis of this technique was performed by (Joshi and Fernandez 1988) and the improvements were published by (Lindau and Neher 1988, Fidler and Fernandez 1989, Okada et al. 1992). Some drawbacks of these methods (see below) were overcome by introducing a dual frequency method by Rohlíček and Rohlíček (1993) and this method was used for measuring changes of the cell membrane capacity of pancreatic acinar cells (Rohlíček and Schmid 1994). The method was later applied and published by Donnelly (1994), improved by Rech et al. (1996) and modified by Barnet and Misler (1997).

The measurement of cell membrane electrical parameters (i.e. membrane capacity ($C_m$) and membrane resistance ($R_m$) or membrane conductivity ($G_m$) ) most often employs a patch clamp arrangement in the whole cell configuration. The measurement of electrical membrane parameters is frequently combined with the patch clamp measurement and for both these measurements the same input probe is used. An operational amplifier with a negative feedback in the probe transforms the current flowing through the access resistance and membrane into a proportional potential. It will be shown that at least three parameters of this...
configuration (feedback stray capacity, open loop gain and transient frequency of the amplifier) negatively influence the proportionality between the input current and the output potential as far as the amplitude and phase are concerned. This causes a degradation of the accuracy of the evaluation as well as of the suppression of the mutual influence between the individual measured parameters which can introduce artifacts.

**Fig. 1.** Arrangement of the measurement.

**Methods**

Figure 1 presents a typical arrangement for the measurement. The timer uses a crystal controlled oscillator for generation of the following signals: control signals for the AC generator, switching potentials for synchronous detectors, control signals for the computer and an A/D transducer and multiplexer. The signal from the AC generator is applied to the cell and the current/potential conversion is performed in the input amplifier (block „Measured cell - Input probe in Fig. 1). A detailed scheme of this part is presented in Fig. 2. The synchronous detectors offer potentials proportional to the real and imaginary components of the output of the probe. At the input of the measuring apparatus (probe) an i-u (current - potential) transducer is used. In most cases a probe of a patch clamp device serves as this transducer.

**Fig. 2.** Possible input combinations, a) inverting combination, b) non-inverting combination, c) transformation of input admittance. \( R, C = \) transformed input admittance, \( ac = \) ac generator, \( Cv = \) input capacity of the i/e converter, \( Ao, ft = \) internal constants of the i/e converter, \( Cf, Rf = \) feedback parameters of the i/e converter, \( dif = \) differential amplifier.

Figs. 2a and 2b offer two possibilities of the connection of the input probe. The solution 2a is more simple (a differential amplifier is not necessary). Its disadvantage is that an ac potential in the bath requires an ac source with minimal internal resistance. The advantage of the connection 2b is the grounded bath. In most connections of this type an input dual FET transistor serves as a differential amplifier with emitters connected to a current source (Sigworth 1983). We assume that the input capacity is compensated in both cases.

An admittance formed by any combination of resistors and capacities connected to the input terminals \( a \) and \( b \) (Fig. 2c) can be expressed with real \( (Re) \) and imaginary \( (Im) \) components. For the special case of a serial connection of a resistor \( (Ra) \) with a parallel combination of the resistor \( Rm \) and a capacity \( Cm \) which is typical for measurements of electrical cell membrane parameters, both components are given by the following equations:

\[
Re = \frac{1}{Rm} \left( \frac{1}{Rm} + \frac{Ra}{Rm + \omega^2 Cm^2 Ra Rm} \right)
\]

(1)

\[
Im = \frac{\omega Cm}{\left( \frac{1}{Rm} + \frac{Ra}{Rm} \right)^2 + \left( \omega Cm Ra \right)^2}
\]

(2)
where: $Ra =$ access resistance, $Rm =$ membrane resistance and $Cm =$ membrane capacity $Re$ is the real part, $I/Re$ then the transformed resistance $R$ and $Im$ the imaginary part of the admittance between terminals $a$ and $b$, the transformed capacity $C$ equals $Im/\alpha$. Note that the transformation is valid only for one particular frequency.

A potential proportional to this admittance occurs at the output of the $i/u$ transducer only under the assumption that the input capacity is compensated, there is no stray capacity in the feedback loop ($C_f=0$), the amplification and transient frequency of the amplifier are so high, that their influence on the $i/u$ conversion can be neglected and that the input capacity is compensated.

Under real conditions, when a commercial patch-clamp device is used for the input capacity compensation, any other compensations have to be switched off. The real component $Re$ and imaginary component $Im$ of the admittance both change at the output of the $i/u$ transducer in $I$ (inphase component) and $Q$ (quadrature component). A $dc$ potential proportional to $I$ and/or $Q$ then occurs at the output of synchronous detectors the switching signal of which has a phase relation to the excitation potential strictly $0$ or $\pi/2$.

These expressions are valid for a noninverting connection. For an inverting connection only $\alpha$ and $\beta$ changes into:

$$\alpha = R_f . Re$$

$$\beta = R_f . Im/\omega$$

The presence of the frequency $\omega$ in the equations signifies that the correction is valid only for one given frequency. This must be taken into account by the application of dual or multifrequency methods.

$Re$ and $Im$ defined by (1) and (2) are simultaneous variables in a system of two non-linear equations:

$$F(Re, Im) \equiv I - Io = 0$$

$$G(Re, Im) \equiv Q - Qo = 0$$

where $Io$ and $Qo$ are the measured values (outputs from the synchronous detectors). $I$ and $Q$ are defined by equations (3) and (4) and represent a mathematical model of real and imaginary parts of the transfer under real conditions.

Solution for $Re$ and $Im$ of non linear equations (9) and (10) can be performed only by an approximation to:

$$Re_k = Re(11)$$

$$Im_k = Im(12)$$

where $Re_k$ is the real component of the measured admittance after correction, $Im_k$ is the imaginary component of the measured admittance after correction.

The solution has to fulfil the following condition:

$$|F| + |G| \leq \epsilon$$

where $\epsilon$ is small (we have used $\epsilon = 10^{-10}$).

Such a system of non-linear equations can be solved by several methods (Ralston 1973, Nekvinda 1976, Vitásek 1987).

We have selected the Newton method, because we believe that this method has the smallest number of iteration steps for our purpose due its quadratic convergence and thus saves computer time. The computer model has shown, that in most cases the number of iteration steps is 3, rarely 2 or 4.

For the first step of the solution it is necessary to estimate the approximate values of $Re$ and $Im$.
Instead of beginning with fixed values of $Re$ and $Im$ in the centre of the range, we found that the nearest approximation is to use the values given by the output of the synchronous detectors $I$ and $Q$.

The reconstruction of the original parameters $Ra$, $Rm$ and $Cm$ depends on the method employed. The methods which use one sinusoidal frequency underestimate the system because only two equations are at our disposal for the three variables. Therefore, a third relation has to be found. For instance, a $dc$ potential was superposed on the sinusoidal potential (Lindau and Neher 1988). This technique assumed a constant and known reversal potential of the cell during the whole experiment. Possible electrical stimulation of the cell by a $dc$ component of the excitation potential cannot be excluded. A phase tracking technique published by (Fidler and Fernandes 1989) assumes that the cell parameters are approximately constant during the experiment. Another approach was the superposition of a slow (2 Hz) rectangular potential to the sinusoidal measuring potential (Okada et al. 1992). This technique does not allow registration of abrupt changes of the membrane parameters. The mathematical derivation of the original parameters is described in papers mentioned above.

To overcome the drawbacks of monofrequency methods a dual frequency method was developed (Rohlícek and Rohlíček 1993). We applied the correction described for this method. In this case, it was necessary to correct the output of the synchronous detectors for both frequencies.

To obtain the original parameters the following equations were used:

$$Cm = c \cdot \frac{k \cdot a_z \cdot (a_z^2 + a_z^3) - a_z \cdot (a_z^2 + a_z^3)}{(1 - k) \cdot \omega \cdot a_z}$$

$$Rm = \frac{1}{c} \cdot \frac{(k - 1) \cdot a_z \cdot a_z}{a_z \cdot (a_z^2 + a_z^3) - a_z \cdot (a_z^2 + a_z^3)}$$

$$Ra = \frac{1}{c} \cdot \frac{(k \cdot a_z \cdot a_z - a_z \cdot a_z)}{k \cdot a_z \cdot (a_z^2 + a_z^3) - a_z \cdot (a_z^2 + a_z^3)}$$

where $a_1 - a_4$ are the output potentials of the synchronous detectors ($ReI$ and $ImI$); $a_1$ is a real and $a_2$ an imaginary component for the low frequency, $a_3$ is a real; $a_4$ an imaginary component for the high frequency; $\omega$ is the low frequency and $k$ is the ratio of both frequencies; $c$ is a coefficient of proportionality respecting the value of the measuring potential and the amplification in the evaluating chain.

**Results**

The tests were performed on a) a computer model and b) a model circuit. Both were used for testing all combinations of values $Rx = 1, 2, 4, 8 \, M\Omega$, $Rm = 100, 200, 400, 800, 1600 \, M\Omega$, $Cm = 5, 10, 15 \, pF$ (which, as we believe, cover the relevant range).

a) The computer model allowed us not only to verify the mathematical procedure, but it also made it possible to investigate rapidly and accurately the influence of an incorrect estimation of any component in the feedback loop, as well as of the parameters of the amplifier.

b) As a model circuit, the connections shown in Fig. 2a and 2b were constructed with scaling of the resistances $1/1000$ and capacities $1000/1$. This arrangement of the model was used for the following reasons:

1. It would be nearly impossible for the original values, in the range of interest mentioned above, to construct a switching arrangement by which changes of the capacity during switching would be smaller than $5 \, fF$ (including differences in the stray capacity on individual components).

2. An accurate calibration of the component would only be possible by using special measuring devices.

3. The noise by the scaling described above is much smaller and therefore the measurements are more accurate.

The comparison with values measured with the model circuit have shown good agreement with the measurements on cells.

The application of the method described requires knowledge of the values of the feedback components and parameters of the amplifier. In the technical data summarized in the handbook delivered along with the device, only values of the feedback resistance $Rf$ are reported. Other values, i.e. feedback capacity, open loop gain and transient frequency of the amplifier have to be estimated. The most frequent artifact when measuring the cell membrane capacity is the influence of $Cm$ indicated by changes in cell membrane resistance which can easily exceed 100% (Rohlícek and Schmid 1994). Therefore, we paid attention to the problem how the incorrect estimation of the feedback capacity $Cf$, open loop gain of
the amplifier $A_0$ and the transient frequency of the amplifier $f_t$ influence the suppression of this artifact.

For this purpose we used a standard sensitivity function, for example that proposed by Di Stefano et al. (1967).

$$C = \frac{Rm_1}{Cm_1} \left( \frac{Cm_2 - Cm_1}{Rm_2 - Rm_1} \right) \quad (17)$$

We are using the reciprocal value of the sensitivity function, i.e. the ratio of the relative change of resistance $R_m$ to the relative change of capacity $C_m$ which expresses the suppression $S_f$ of the artifact.

The analysis was performed for the following parameters: the low frequency (500 Hz) and high frequency (1 kHz), the open loop gain of the amplifier $A_0 = 10^5$, its transient frequency $f_t = 10$ MHz, the feedback resistance $R_f = 500$ MΩ and the feedback stray capacity $C_f = 100$ pF.

Tables 1 to 3 show how critical is the estimation of the values $C_f$, $A_0$ and $f_t$.

First were calculated in phase and quadrature values in the whole relevant range, i.e. $R_s = 1, 2, 4, 8$ MΩ, $R_m = 100, 200, 400, 800, 1600$ MΩ, $C_m = 5, 10, 15$ pF.

In the second step correction was performed with incorrect setting of $C_f$, $A_0$ and $f_t$ as indicated in the last columns of the tables. All combination of values in the range of interest were taken into account and the worst case conditions were estimated and presented in rows. The odd rows are for positive disagreement from the correct value, the even rows for the negative one.

The last column shows the difference from the proper values of the respective parameters.

The factor $S_f$ illustrates the suppression of the change of membrane resistance to the membrane capacity and is a reciprocal value of the sensitivity function.

The curves in Fig. 3. illustrates the change of the suppression factor as a function of the value of membrane resistance $R_m$ in the middle range, i.e. for capacity $C_m = 10$ pF and access resistance $R_a = 2$ MΩ and disagreement of the capacity $C_f = +/− 1$ and 3 %.

### Table 1.

<table>
<thead>
<tr>
<th>$R_a$ MΩ</th>
<th>$R_m$ MΩ</th>
<th>$C_m$ pF</th>
<th>$S_f$</th>
<th>$\Delta C_f$</th>
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</thead>
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</tr>
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<td>-3 %</td>
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<td>5</td>
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<td>-1 %</td>
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<td>15</td>
<td>458</td>
<td>-1 %</td>
</tr>
<tr>
<td>1</td>
<td>1600</td>
<td>5</td>
<td>5000</td>
<td>+3 %</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>15</td>
<td>517</td>
<td>+3 %</td>
</tr>
<tr>
<td>1</td>
<td>1600</td>
<td>5</td>
<td>2500</td>
<td>+1 %</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>15</td>
<td>263</td>
<td>+1 %</td>
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### Table 2.

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<th>$R_m$ MΩ</th>
<th>$C_m$ pF</th>
<th>$S_f$</th>
<th>$\Delta f_t$</th>
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<td>1500</td>
<td>-20 %</td>
</tr>
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<td>1600</td>
<td>5</td>
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<td>-20 %</td>
</tr>
<tr>
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<td>1600</td>
<td>5</td>
<td>$&gt;10^4$</td>
<td>+50 %</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
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<td>+50 %</td>
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### Table 3.

<table>
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<th>$R_a$ MΩ</th>
<th>$R_m$ MΩ</th>
<th>$C_m$ pF</th>
<th>$S_f$</th>
<th>$\Delta A_0$</th>
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<tr>
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<td>50</td>
<td>15</td>
<td>$&gt;10^4$</td>
<td>+100 %</td>
</tr>
</tbody>
</table>

**Fig. 3.** Decrease of the suppression factor $S_f$ by incorrect setting of the feedback capacity $C_f$. 

\[ S_f = f(R_m) \]
Discussion

Regardless of the method used for measurement of electrical membrane parameters, the measurement starts with calibration of the apparatus. This calibration is mostly performed by connecting the probe with a model circuit (dummy) the electrical parameters of which are known. This calibration includes the compensation of all influences described above. A discussion of the equations (1-4) shows that the distortion introduced by the mentioned parameters of the apparatus depends on the actual values of $Ra$, $Rm$ and $Cm$. In other words, the calibration compensates all influences only for just one particular case. The admittance of the combination of $Ra$, $Rm$ and $Cm$ represent a vector. By a dual or higher frequency method it is necessary to consider admittances as vectors. If another combination occurs the vector(s) are different. The greater the difference, both in the angle as well as in the amplitude of this (these) vector(s), the lower the compensation of the influences mentioned above. This leads to the following results:

a) Inaccuracy of the absolute values of the parameters measured. In most of the real conditions this inaccuracy does not exceed several percent, which is not very important because of the differences of these parameters in different cells of the same type if these cells also belong to the same sample.

b) Of considerable importance is the degradation of the suppression of the influence exerted by the change in membrane resistance on the indicated capacity (suppression factor) in case of another combination compared to that for which the calibration was performed. Such abrupt changes in membrane resistance can exceed 100 % as shown by Rohlíček and Schmid (1994). Taking into account that the changes in membrane capacity of interest (single events of exocytosis, endocytosis, granulation, fertilization etc.) are in the range $10^{-3}$ of $Cm$ (each single event by about 10 fF of the total membrane capacity of 10 pF), the suppression factor has to be at least 1000, otherwise there arises danger of artifacts which cannot be neglected.

The above method has the following advantages:

a) Only one calibration is necessary which is valid for all input admittances without loss of the suppression factor thus minimizing the artifacts.

b) No model circuits are necessary for the calibration. It is sufficient to connect the inputs of the synchronous detectors with the output of the generator of measuring potentials and to set the in-phase potential to 1 and quadrature one to zero.

As mentioned above, it is necessary for the application of the method to insert the values of $Rf$, $Cf$, $Ao$, $ft$ into the equations. In the technical data of the patch-clamp apparatus only $Rf$ values are usually given.

When looking at Tables 1, 2 and 3 it is possible to conclude that the open loop amplification $Ao$ has the smallest influence on the correction process and that in most cases we may assume this value to be $5.10^4$ without introducing a significant error. The estimation of the transient frequency is more important and at least a rough assessment is necessary. The accurate estimation of the feedback capacity is of paramount importance. Table 1 shows that an accuracy better then 3 % is desirable. The same accuracy has to apply to the value of $Rf$. In this case, a verification of the value published in the manual is to be recommended. In the Appendix, some of the methods for measurement of the mentioned parameters are described.

During practical application of the system (Rohlíček and Schmid 1994) a negative influence of distributed parameters of the pipette was not observed.

The mathematical derivation of the most equations in this paper would be out of the scope of this journal. A theoretical paper with the complete mathematical derivation is under preparation. The readers interested in this part of the present communication should contact the senior author of this presentation (V. R.).

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Appendix

Measurement of the feedback capacity $Cf$ and the parallel input capacity $Cp$

The most important parameter for the correction software is the value of the feedback capacity $Cf$. A direct measurement is almost impossible, because the greatest part of this capacity forms the stray capacity of the feedback resistor $Rf$ which is in most cases an intrinsic part of the customer-integrated circuit in the probe. We developed a method for the indirect measurement of this capacity which also allows the estimation of a parallel input capacity ($Cp$).

This method only uses one frequency, so that it can be used for all ac methods for measuring the
electrical parameters of the cell membrane. For these measurements, an auxiliary circuit consisting of a parallel combination of a capacity ($C_1$) and a resistor ($R_1$) is connected to the input of the probe (points $a$ and $b$ in Fig. 2a and 2b.). The estimation of $C_f$ and $C_p$ is based on the solution of the following equations (Ralston 1965, Nekvinda 1976).

$$F(x,y) \equiv I - Io = 0$$  \hspace{1cm} (18)

$$G(x,y) \equiv Q - Qo = 0$$  \hspace{1cm} (19)

where $I_0$ and $Q_0$ are measured inphase and quadrature components, $x$ and $y$ are auxiliary variables given by

$$x = Rf.(C_1 + C_p), \Rightarrow C_p = x/Rf - C_1$$  \hspace{1cm} (20)

$$y = Rf.C_f \Rightarrow C_f = y/Rf$$  \hspace{1cm} (21)

The solution of equations 18 and 19 is performed by the Newton method (Vitásek 1987). The following expression is used as the first approximation:

$$x_0 = (I_0^2 + Q_0^2)/(\omega \cdot AB SQ_0); \ y_0 = I_0/(\omega \cdot AB SQ_0)$$  \hspace{1cm} (28)

Because this measurement is only performed once for a given apparatus, it is possible to compare the values after averaging 100 times.

**Measurement of the feedback resistor $R_f$**

The value of the feedback resistor $R_f$ is of similar importance as the value of the feedback capacity $C_f$ because for the accurate correction they represent together the feedback impedance (or feedback time constant $\tau_f = C_f \cdot R_f$).

The value of this resistor is mostly given in the data sheet of the patch-clamp device, however, usually without the required accuracy of this value.

Fortunately, the measurement of the feedback resistor $R_f$ is relatively easy. It is sufficient to connect an auxiliary resistor $R_{aux}$ of a known (accurate) value to the input terminal of the probe and the other terminal of the resistor to a known $dc$ potential and measure the output potential of the $i/e$ transducer. $R_f$ is then given by:

$$R_f = R_{aux} \cdot u_{in} / u_{out}$$

where $R_{aux}$ is the auxiliary resistor $u_{in}$ the known $dc$ potential $u_{out}$ the output potential of the probe.

**Measurement of the transient frequency $f_t$.**

The direct measurement of the utilize a frequency $f_t$ is, not possible owing to the unavoidable capacity $C_f$. Therefore, we can use the property of an operational amplifier the amplifying factor of which decreases with the frequency from the open loop gain $A_0$ to the factor 1 with a slope of -20 dB/dek (Dostál 1981).

For a negative feedback, the amplification is given by:

$$A = R_f / R_1 \text{ inverting input,}$$

$$A = 1 + R_f / R_1 \text{ non - inverting input.}$$
$R_f$ is the feedback resistor between the input and output terminals of the amplifier and $R_i$ is a resistor connected between the terminal of the amplifier and zero potential.

It is sufficient to estimate the frequency for the 3 dB point (corner frequency $f_c$). The transient frequency is as follow:

$$f_t = f_c \cdot A$$

A small value of the resistor $R_f$ should be selected compared to the feedback capacity $C_f$.

Table 2 shows that this measurement is not very critical, an error of 20% is acceptable.

**Measurement of the open loop gain $A_o$**

Most methods for this measurement utilize a resistance divider $R_3 / R_4$ (Fig. 4.). If $R_1 = R_2$ the open loop gain is:

$$A_o = \left(\frac{R_3}{R_4} + 1\right) \frac{e_{out}}{e_d}$$

In most marketed devices it is not possible to insert the divider $R_3 / R_4$. In this case ($R_3 = 0, R_4$ omitted) the potential $e_d$ is equal to the potential between the input terminals of the amplifier $e_{in}$. If $R_1 = R_2$ the open loop gain is given by:

$$A_o = e_{out} / e_d$$

To exclude the influence of the feedback capacity (not drawn), the measurement should be performed either with a dc potential or with an ac potential the frequency of which is below the first corner frequency of the amplifier.

![Fig. 4.](image)

**References**


**Reprint requests**

V. Rohliček, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic. E-mail: Rohlicek@biomed.cas.cz