—Short Communications—

Musing of the feline hepatic voltage-dependent delayed-rectifier potassium channel gating

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The ion transport mechanisms across the liver cell plasma membrane have been characterized in a variety of preparations from different species by measuring intracellular ion concentrations and net isotope fluxes. The hormonal stimulation-induced increase of K^+ permeability was demonstrated using intracellular recording in guinea-pig liver slices. Recently, the patch-clamp technique has been applied to examine membrane ion conductances in mammalian hepatocytes and we have previously shown the delayed-rectifier K^+ channel characteristics in isolated guinea-pig hepatocytes using the whole-cell and single-channel recording techniques¹. The current was modulated by the intracellular Ca^{2+} without affecting the voltage-dependence and was suppressed by external application of 4-AP or apamin. In contrast, it is known that rat hepatocytes have ohmic conductance in the current-voltage (I-V) relationship. We report here the voltage-gated single K^+ channel characteristics in isolated feline hepatic cell membranes.

Feline hepatocytes were enzymatically dissociated in a manner similar to that described previously^{2,3}. Cats were anaesthetized with either sodium pentobarbital (42.0 mg/kg body weight, i.p.) or ketamine HCl (11.0 mg/kg body weight, IM) and the portal vein was cannulated. Liver perfusion was initiated with a Ca^{2+} -free solution (first perfusion solution) for 10 min at a flow rate of 5 ml/min. The perfusate was switched to a solution containing 4 mM Ca^{2+} and 0.5 mg/ml of collagenase (Sigma, type I, St. Louis, MO, USA) for 10 min at 10 ml/min (second perfusion solution). Following these perfusions, the liver was excised and then minced in Hank's balanced salt solution (BSS). The cells were

filtered through a 150- μ m nylon mesh, and washed 3 times by centrifugation at $100\times$ for 5 min. The cell pellets were resuspended in Hank's BSS and used within 5 hours following the isolation. The first solution perfused via the portal vein cannulation contained (in mM): NaCl 140, KCl 5.4, NaH_2PO_4 0.6, Na_2HPO_4 0.4, EGTA (ethylene glycol-bis-(β -aminoethylether) N,N,N',N',-tetraacetate) 0.5, $NaHCO_3$ 4.2, glucose 5, and N'-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-NaOH buffer 9.6, brought to pH 7.2 with NaOH. The second perfusion solution contained (in mM): NaCl 140, KCl 5.4, $CaCl_2$ 4.0, NaH_2PO_4 0.6, Na_2HPO_4 0.4, $NaHCO_3$ 4.2, HEPES-NaOH buffer 9.6, and collagenase 0.5 (pH=7.5). Hank's balanced salt solution (BSS) contained (in mM): NaCl 140, KCl 5.4, $CaCl_2$ 1.3, $MgCl_2$ 1.0, $MgSO_4$ 1.7, NaH_2PO_4 0.6, Na_2HPO_4 0.4, $NaHCO_3$ 4.2, glucose 5 (pH=7.2). The external normal Tyrode's solution contained (in mM): NaCl 142, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, glucose 10, HEPES 8 (pH=7.3). Pipette solution (standard internal solution) contained (in mM): KCl 150, $MgCl_2$ 1.0, EGTA 5, HEPES 8 (pH=7.3 with KOH). Temperature of the bath was monitored and was maintained at $37 \pm 1^\circ C$ using a Peltier thermo-electrical device. Membrane currents were recorded using the patch-clamp technique and an Axopatch-1C amplifier (Axon Instruments, Inc., Foster City, CA)^{4,5}. Inverted voltage-clamp pulses were applied to the bath through an Ag-AgCl pellet-KCl agar bridge with the pipette potential maintained at ground level. The head stage of the voltage-clamp circuit was set up with an ultra-low bias current operational amplifier. The feedback resistance of the head stage was 50 G Ω for

recording single channel current. The output of the voltage-clamp amplifier was adjusted to give zero current when the tip of the pipette (filled with internal solution) was immersed in the bath containing control external solution⁶⁻⁸. Currents were monitored with a storage oscilloscope (Type 5113, Tektronix, Inc., Beaverton, OR) and were stored continuously on digital audio tape (R-60 DM, MAXELL, Tokyo, Japan) using a PCM data recording system (RD-100 T, TEAC, Tokyo, Japan)⁹. The single channel events were reproduced and filtered off-line with a cut-off frequency of 2 KHz through an eight-pole low-pass Bessel filter (48 dB/octave, Model 902-LPF, Frequency Devices, Inc., Haverhill, MA), digitized with 14-bit resolution at a sample rate of 10 KHz^{10,11}. Leakage currents during voltage step recording were canceled by subtracting each trace from the average current traces without events. The data were analyzed on a computer (PC-9801, NEC, Tokyo, Japan) using locally written analysis programs that are based on the half-amplitude threshold analysis method¹²⁻¹⁴. Channel transitions were calculated using an averaging technique for determining channel amplitude. The results are expressed as mean $\pm$ SD. Differences between sample means were determined using Student's t-test or one-way analysis of variance. A p value <0.05 was considered statistically significant.

Two-second duration of test pulses were applied to +60 mV from holding potential of -80 mV to record single channel activity in the outside-out patch configuration. The depolarizing voltage steps to +60 mV caused multiple channel openings after a latency period. Channel activity exhibited bursting behavior. The channel open events in the individual patch increased with time resulted in the enhance-

ment of open channel activity. In contrast, neither unitary current amplitude nor mean open lifetime during the voltage-clamp step changed significantly. The ensemble current obtained from 200 consecutive sweeps at +60 mV demonstrated the time-dependent increase in current amplitude. Open time distribution was best described by a single exponential function with the mean open lifetime of 20.4 ± 2.7 ms ($n=11$). At least two exponentials were required to fit the closed time distributions with a time constant for the fast component of 1.1 ± 0.3 ms ($n=11$) and that for the slow component of 51.8 ± 6.6 ms ($n=11$). The gating kinetics of the channel resembles that in previously reported in guinea-pig preparations¹. Single channel behavior exhibited bursting opening behavior separated by interburst periods. Although neither the mean open lifetime nor shut period during a burst showed significant voltage-dependence, shut periods between bursts increased with less depolarization of the membrane voltage. According to the dwell time histograms of open and closed time distributions, channel state can be described by a linear sequential gating scheme having one open and two closed states:

where C_1 and C_2 are closed states, O is the open state, $k+1$ and $k-1$ are rate constants between C_1 and C_2 states and α and β are rate constants between C_2 and O states. Each rate constant was calculated from a function of the dwell time constants and open probability at the given voltage using the two closed states gating scheme. Averaged rate constants at +60 mV from 11 different patches were $\alpha=51.4 \pm 6.0$, $\beta=488.0 \pm 61.5$, $k-$

Table 1 Voltage-dependence of the rate constants

Voltage(mV)	α	β	$k-1$	$k+1$
+60	51.4 ± 6.0	488.0 ± 61.5	62.7 ± 7.3	19.9 ± 4.8
+40	69.5 ± 6.1	514.8 ± 67.8	70.0 ± 6.9	19.0 ± 5.2
+20	94.2 ± 7.7	902.3 ± 84.1	141.8 ± 10.3	12.5 ± 4.9
0	114.7 ± 12.8	995.4 ± 90.3	162.4 ± 12.8	5.5 ± 2.0

Rate constants were calculated in terms of a linear sequential gating scheme at different test voltages between +60 and 0 mV. α and β are rate constants (s^{-1}) between C_2 and O states and $k+1$ and $k-1$ are rate constants (s^{-1}) between C_1 and C_2 states. All rate constants were expressed as mean $\pm$ SD from 11 different outside-out patches.

Table 2 Comparison of the observed (experimental) and predicted burst lengths at different voltages

Voltage (mV)	the observed burst length(ms)	the predicted burst length(ms)
+60	241.8±39.1	207.5±44.1
+40	201.4±32.5	180.9±40.2
+20	163.8±31.1	145.2±25.7
0	114.0±29.9	82.3±19.0

The observed burst length was averaged from seven different outside-out patches. The predicted burst length was calculated from rate constants shown in Table 1 (n=11). Data were expressed as mean±SD. No significant differences were detected between the two values in each voltage.

$1=62.7 \pm 7.3$, $k+1=19.9 \pm 4.8 \text{ s}^{-1}$. **Table 1** summarizes the rate constants in different test voltages between +60 and 0 mV. Decreasing the rate constant of $k+1$ appears to be responsible for the voltage-dependent increase of the time constant for the slow component of closed time distributions which would cause the prolongation of the closed period and decrease of channel P_0 . By using these rate constants, the probability density function to describe apparent burst behavior was obtained and the mean apparent burst length was calculated as $207.5 \pm 44.1 \text{ ms}$ (n=11) at +60 mV. Individual bursts contain 5-13 open events interspersed with brief shunt periods. **Table 2** summarizes the comparison between the observed (experimental) and the predicted burst length calculated using the rate constants in **Table 1** in voltages between +60 and 0 mV. They were not different significantly at each

test voltage. The close agreement between the experimental and the predicted burst length supports the linear sequential gating scheme with two closed states and single open state in the feline hepatic delayed-rectifier K^+ channel.

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