developed, in which field potential (FP) of cardiomyocytes in geometrically patterned chambers have been recorded with a multielectrode array (MEA) system. We have also developed micropatterned microstructures on the electrode with same closed circuit shape assisted by agarose microstructures. After the confirmation of regular propagation of beatings, we added a 1 μM Astemizole which is well known to raise ventricular arrhythmias, and found that the profile of FP changed to the abnormal beating shape and then reached to the fibrillation shape [1]. To investigate the propagation pathway of excitation in abnormal beating, we constructed the circuit on 16 electrodes with area of 50x50 μm in a similar geometry to ring electrode assay. When abnormal beating happened on convoluted FPs obtained from all the 16 channels, one directional block on propagation were occurred and induced “re-entry” [2]. These results suggest that abnormal propagation, such as re-entry, causes abnormal fibrillation-like signal on the ring electrode. In conclusion, a simple quasi-normal propagation pathway of FPs obtained from all the 16 channels, that one directional block on propagation were occurred and induced “re-entry” [2]. These results suggest that abnormal propagation, such as re-entry, causes abnormal fibrillation-like signal on the ring electrode.

In contrast, the in vivo ECG measurement assay has been proposed and developed and the results showed the typical arrhythmia profiles, in which both the temporal depolarization information and the spatial beating propagation information were appeared.


Smooth & Skeletal Muscle Electrophysiology

2772-Pos Board B542
Targeting and Functional Expression of Transgenic Chloride Channels (CIC-1) in Skeletal Muscle Fibers of Adult Mice

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The relative distribution of CIC-1 between the surface and transverse tubular system (TTS) membranes in skeletal muscle fibers is still a matter of debate. A useful tool to investigate this issue is to transfect plasmids coding for fluorescently-tagged transgenic channels into the fibers of FDB muscles (by in vivo electroporation), and assess the intracellular targeting of the expression products. Using a human clone (hCIC-1), we recently observed expression of EYFP-hCIC-1 at both the TTS and surface membranes, while another group, using a murine clone (mCIC-1) reported expression of EGFP-mCIC-1 only at the sarcolemma. Since these differences could have originated from divergent experimental conditions, we compared the expression of both clones under identical conditions. The hCIC-1 (NM_013491.2) and mCIC-1 (NM_013491.2) were subcloned in rBCMV and pCDNA3.1 vectors, respectively. The localization of tagged-CIC-1 was assessed by using two-photon laser scanning microscopy. As sarcromeric localization markers we used a second harmonic generation (SHG) band that marks the location of myosin, and di-8-ANEPPS fluorescence that marks the location of TTS. Action potentials and chloride currents were recorded from dissociated FDB fibers using a two-microelectrode system. We found that, as reported previously with hCIC-1, mCIC-1 is targeted to both the TTS and surface membranes. In addition, the overexpression of either CIC-1 clone resulted in significant reductions in the input resistance, and a corresponding significant increase in the chloride currents, recorded from the muscle fibers. Overall, our results demonstrate that transgenic CIC-1 are functionally expressed at the surface and TTS membranes of skeletal muscle fibers. We thank Drs. J. Lueck, U. of Iowa, and C. Fahike, Medizinischen Hochschule Hannover, Germany, for sharing hCIC-1 and mCIC-1, respectively. This work was supported by NIH grants AR047664, AR041802, and AR054816.

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Modification of Women Uterine Contractile Activities by Chronic T4-Treatment is Related to Changes in Ca++ and K+ Conductance: Clinical to Bench Side Analysis

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Significant time course modifications of uterine contractile activities were observed in Levothyroxyrine-treated pregnant women, including increased amplitude and duration of phasic contractions in vitro. This study aimed to investigate the involvement of Ca++ and K+ conductance in controlling repetitive contractile activities in an in vitro model of human uterine strips. Thus, the ability of Ba2+- and Bay K8644 to modulate Ca++ and K+ conductance was used to alter the pattern of control uterine contractile activities. Uterine biopsies were performed from consenting women undergoing elective caesarean sections at term (N=12). Isometric tension measurements were performed in vitro on fresh human myometrial strips in isolated organ baths. After a 2 h equilibration period, the effects of Iberiotoxin (IBTx), a selective BKCa blocker, Ba2+- and Lemakalam, a potassium channel opener were assessed on spontaneous uterine contractile activities. Uterine contractile activities were quantified by calculating the amplitude, the duration, the frequency and the area under the curve over 20-min periods. Our data demonstrated a significant increase in amplitude and duration of phasic contractions following addition of 2 mM Ba2+-, partially mimicking the result obtained upon T4-treatment during pregnancy. Addition of 100 nM IBTx, slightly increased these parameters, similarly to acute T3 treatments (p<0.05). Use of 1 μM Lemakalam efficiently abolished in vitro uterine contractions. Following 2 mM Ba2+-, cumulative addition of Lemakalam produced relaxing effects (p<0.05). Moreover, in the presence of a hypokalemic physiological solution containing 1 mM KCl (as opposed to 4.7 mM) a tocolytic effect was quantified, an effect likely related to enhanced K+ conductance. Our data suggest that in uterine strips, modifications of GK+ could alter the contractile pattern, which would explain the abnormal time course of uterine repetitive contractions.

2774-Pos Board B544
The Effects of Ions and Water on Actin-Mycosis Binding Energies and Mechanics

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Myosin’s discrete lever arm rotation upon strong actin binding is capable of generating large forces, but in ensemble myosin systems the average force generated by this transition is limited by the free energy for actin-mycosis binding. Yet when looking for insights into mechanisms of muscle force generation, myosin structures are studied while factors that affect actin-mycosis binding energetics are seldom considered. Here we use in vitro motility assays, AM binding assays, and stopped flow kinetics to study the effects of water and KCl on actin-mycosis binding. First, we show that sucrose dramatically inhibits actin-mycosis binding kinetics through a mechanism other than crowding or viscous damping, implying that sucrose has a desolvation effect. Second, we show that the effects of sucrose are ionic strength dependent, having little effect at low ionic strength. Our data support a simple model in which the strength of weak actin-mycosis bonds is reduced by the shielding effect of ions, and sucrose enhances this effect possibly by displacing ordered waters around actin and myosin. Our data suggest that water solvation and ionic shielding may have a significant influence on muscle mechanics.

2775-Pos Board B545
Validation of XE991 use as a Selective, Irreversible Blocker of Native Kv7 Channels in Smooth Muscle Myocytes

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A concern was recently raised about the selectivity of XE991 and its use as a Kv7 channel blocker in single cell electrophysiology and in vitro functional assays. In the present study, we evaluated the effects of XE991 on different components of the endogenous delayed rectifier potassium currents recorded in freshly-isolated guinea pig airway myocytes having either 1 or 2, Bela Joos1, Andre Longtin1.

Conductance:

2923-Pos Board B546
Spontaneous Excitation Patterns and Propagation Irregularities Computed For Myelinated Axons with Injury-Like Impairments of Nodal Na/K Pumps and Sodium Channels

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The healthy sodium channel (Nav)-rich node of Ranvier axolemma is a precision, far-from-equilibrium entity, and its nano-structure, not surprisingly, is vulnerable to multiple perturbations, including hypotonic challenge, ischemia, and inflammation, all of which injure plasma membranes through combinations of blebbing, ATP-depletion, high-Ca\(^{2+}\) and reactive oxygen species. Injury-related dysfunction ranges from subthreshold oscillations (STO), to ectopic excitation, to propagation block. We showed that blebbing injury to recombiant Nav-bearing membranes (the nodal isoform Nav1.6) results in a coupled hyperpolarizing-shift of activation and availability, and computationally, we showed that the expression of non-1:1 transmission) along a 7-node axon whose middle node was injured, were explored with no input at the initial segment, with constant current input and with pulses that follow a Poisson distribution of arrival times.

**Estimation of Population-Specific Synaptic Currents from Laminar Multielectrode Local Field Potential Recordings**

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Multielectrode array recordings of extracellular electrical field potentials along the depth axis of the cerebral cortex is an up-and-coming approach for investigating activity of cortical neuronal circuits (Einevoll et al., J. Neurophysiol., 2007; Blomquist et al., PLoS Comp. Biol., 2009). The low-frequency band of extracellular potential, i.e., the local field potential (LFP), is assumed to reflect the synaptic activity and can be used to extract the current source density (CSD) profile. However, physiological interpretation of CSD profile is uncertain because it does not disambiguate synaptic inputs from passive return currents or identifies population-specific contributions to the signal thus obscuring its interpretation in terms of the excitation flow in the columnar microcircuit.

Here we present a novel anatomically informed model for decomposing the LFP signal into population-specific contributions and for estimating the corresponding laminar profiles of synaptic inputs. This involves a combination of 1) the linear inverse model which predicts population-specific laminar LFP in response to synaptic inputs applied to the population of cells having realistic morphologies; and 2) the linear inverse model, which reconstructs laminar profiles of synaptic inputs from laminar LFP data based on the forward prediction. Assuming spatial correlation of synaptic inputs within individual populations, the model decomposes the columnar LFP into population-specific contributions and estimates the corresponding synaptic input laminar profiles less the mean value. Constraining the solution with a priori knowledge of the spatial distribution of synaptic connectivity further allows estimating the strength of active synaptic projections from the columnar LFP profile thus fully specifying synaptic inputs.

The capability of the model is demonstrated by applying it to the experimental extracellular data.

**Volume Control in the PVN: A Role for TRPV4**

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The hypothalamus is responsible for maintaining body fluid osmolarity within a narrow range (~290-300mOsm)[1]. Upon hypotonic challenge, increased activity and density evolution of the neuronal oscillator. To construct the Markov operator, we use the asymptotic expansion of stochastic processes. We show that the second eigenvalue of the product of the Markov operators reflects a narrow range (~290-300mOsm)[1]. Upon hypotonic challenge, increased heart rate, blood pressure and renal sympathetic activity have been seen[2]. The paraventricular nucleus (PVN) has been implicated as having a major role in osmoregulation. Treatment with hypertonic solutions has shown increases in action potential (AP) frequencies within parvocellular neurons[3]. The transient receptor potential vanilloid channel TRPV4 is a possible candidate for volume sensing within the PVN as it has this role in other tissues [4].

APs were recorded in brain slices using cell-attached patch clamp electrophysiology to investigate the mechanisms of osmoregulation within the parvo-cellar PVN. Results are given as a normalised mean ± SEM, significances were assessed by paired t-test. We also modelled the action of TRPV4 in Neuron (University of Yale) to determine if activity of this channel is likely to account for changes in AP frequency.

We investigated the effects of hypertonic challenge (280mOsm) on AP frequency within the PVN; AP frequency decreased significantly from 1.0 ± 0.1 Hz to 0.5 ± 0.1 Hz (n=7, p<0.01). The role of TRPV4 was investigated using the agonist 4a-phorbol 12,13-didecanoate (4a-PDD). Application of 1μM 4a-PDD decreased AP frequency to 0.8 ± 0.51Hz (n=6, p<0.05), suggesting the presence of TRPV4 channels in neurons within the PVN.

Further investigation was carried out using the TRPV4 specific antagonist RN1734 (5μM). Slices were subjected to hypotonic challenge to ascertain if the patched cell responded and the slice was then treated with RN1734 to inhibit this response. AP frequency decreased during hypotonic challenge to 0.4 ± 0.06 Hz and returned to 0.71 ± 0.17Hz upon treatment with RN1734 with no significant difference between AP frequency before hypotonic challenge and with treatment of RN1734 (n=3; p>0.05). These results suggest TRPV4 within the neurons of the PVN is involved in volume sensing.