行政院國家科學委員會補助專題研究計畫成果報告

在人體身上量測週邊神經軸突納離子通道功能

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Measurement of Sodium Channel Function of Human Peripheral Nerves in vivo

This study was performed to set up a clinical test to evaluate the function of axonal sodium channels in vivo. The latent addition (LA) test was controlled by the “QTRAC” system. Recordings were made by a surface disk electrode on abductor digiti minimi, or by a concentric EMG needle electrode inserted in first dorsal interosseus muscle for the single axon study. Differences in threshold changes between the depolarizing conditioning and the hyperpolarizing one were calculated to represent the axonal local response, which in terms reflect the subthreshold sodium channel current. This tracking strategy was also applied to lidocaine-treated rats. Results of single axon LA showed that the local response was inversely correlated to the axonal threshold. An average response of 10 units from one ulnar nerve was equal to the response obtained by multiunit surface recording, suggested that the multiunit preparation was reliable to estimate the local response of peripheral nerves. In animal study, the local response of rat nerves significantly diminished after the injection of lidocaine confirmed that the local response was sodium channel dependent. We concluded that the LA test is a reliable, non-invasive, easy-performed tool for evaluating sodium channel function in human axons.

Materials and Methods

Latent Addition of Single Motor Axons

Procedures of latent addition test was
similar to that has been reported (Bostock, Rothwell, 1997), except the recording method and the target for threshold tracking. In brief, stimulation on the ulnar nerve was given through a pair of non-polarizable surface disk electrode. The cathode was on the wrist part of the ulnar nerve and the anode on a remote indifferent area. Electric stimuli, generated by an isolated current generator driven by an IBM-compatible PC running QTRAC software (copyright Institute of Neurology, Queen Square, UK), were given in constant current square waves of 60-µs duration. Eight stimulus conditions: one test-alone stimulus (control), test stimulus added to a conditioning pulse set to 90 (depolarizing), 60, 30, –30 (hyperpolarizing), –60, and –90% of the last control stimulus, and an unconditioned stimulus at various pulse duration (see below for estimation of strength-duration time constant), were tested in turn. Delays between conditioning and the test stimuli were from –0.2 (the test pulse given before the conditioning pulse) to 0.5 ms in 20-µs steps.

A concentric EMG needle was inserted in the first dorsal interosseus muscle to record motor unit potentials. Signal obtained from the EMG electrode was fed into a differential amplifier having a band-pass filter of 10 to 10 kHz, and was digitized by an A/D converter (DT2812A, Data Translation, USA) installed in the PC. To avoid baseline wandering in the sweep and eliminate interference peaks originated from distant motor units, a high-pass digital filter at cutoff frequency of 500 Hz, or a first-order differential filter, was applied before the signal was employed for threshold tracking. Before starting the automatic tracking process, stimulus intensity was manually tuned and the position of EMG electrode was carefully adjusted to obtain a sweep in which an isolated, undisturbed single motor unit potential (SMUP) was clearly seen. Current intensity was usually around 10 to 20 mA for this purpose. An SMUP was usually a biphasic wave of which the emergence should follow the all-or-none law.

Target for the threshold tracking was set to half of the peak height of the sampled SMUP. During the threshold tracking, if the SMUP was “all” (a peak cross the tracking target within a predefined window) the current intensity would be reduced by 2% for the next test of the same stimulus condition. On the contrary, if the SMUP was “none” (the sweep contained only the background noise) the intensity would be increased by 2%. When the given current intensity was steadily fluctuated in a small range for a stimulus condition at a specific conditioning-test delay, i.e. the SMUP was alternatively “all” and “none” for a number of trials, the delay time was increased one step. Conditioned threshold for this specific condition was calculated by averaging the given currents in the last few trials. Because there could be a progressive shift in SMUP amplitude due to movement of the electrode tip during the succession of threshold tracking, the tracking target was automatically adjusted to the midway between the amplitude of the most recent peak and the maximal amplitude of the background noise. The threshold tracking process was stopped when every stimulus condition finished the test for 0.5-ms delay.

Calculation of the data was run by the PC. Percentage threshold changes were calculated as 100% × (conditioned threshold – control threshold) / (control threshold) for each stimulus condition at every conditioning-test delay. Threshold changes were plotted against the delays as an eventual result of the latent addition. Asymmetry in the threshold recovery between depolarizing and hyperpolarizing conditionings was calculated by subtraction of hyperpolarizing trace from the corresponding depolarizing one, of which only stimuli having a positive delay (a conditioning pulse given before the test stimulus) were calculated.

**Strength-Duration Time Constant**

Threshold tracking for the
A strength-duration (SD) curve was performed simultaneously with the latent addition. An identical motor axon was sampled in terms of having a same SMUP waveform at an approximately equal latency. Stimulus pulse duration was set from 60 to 1000 µs in steps of 20, 40, or 60 µs. Tracking strategy was the same as that for the latent addition except that the stimulus intensity was corrected by 1% for each “all” or “none”. Axons that can not be studied beyond the pulse duration of 600 µs were discarded, most often due to contamination of other units having a longer strength-duration time constant and a lower rheobase. For possible cases, the SD time constant and the rheobase were determined from Weiss’s formula using plots of threshold charge against stimulus duration (Mogyoros, Kiernan, Burke, 1996).

Nerve Conduction Velocity

Conduction velocity (NCV) of each motor axons was calculated in some possible axons. Immediately after the tracking process was completed and position of the recording electrode was still kept in situ, a proximal stimulation was applied to elbow segment of the ulnar nerve. Stimulus intensity was carefully tuned from a sub-threshold level up to obtain an undisturbed SMUP. An SMUP with the same waveform as that obtained from the wrist stimulation suggested the same motor axon being recruited. NCV was calculated by the latency difference between the proximal and distal stimulation.

Multiunit Latent Addition

A latent addition test in multiunit preparation (Bostock, Rothwell, 1997) was performed on the respective nerve after the single unit study was completed. Compound muscle action potentials (CMAPs) were recorded from the skin over first dorsal interosseus muscle. To compare with the result of single unit studies, the tracking target was set to the amplitude of CMAP that elicited by the stimulus giving intensity equal to the average of control threshold of all sampled axons.

Aminal studies

Adult Wistar rats were studied to verified that the axonal sodium channels were responsible for the result of LA. The rat was anesthetized by intraperitoneal injection of sodium pentobarbital. Four subcutaneous ping electrodes were inserted at proximal part of the tail, arranging in a square to serve as a stimulation device. Recordings of target CMAP was made via a needle electrode inserting in tip of the tail.

LA tests were similar to that described in last section of the multiunit LA. Lidocain 50 mg/Kg was injected into the peritoneal cavity after a control LA test, and follow up LA tests were performed at immediately and 15, 30, 45, 60, 75, and 90 minutes after the injection. The results were calculated as that for the human study.

Results and Discussion

Single Unit LA

Single-unit latent addition test was successfully performed on 46 motor axons from four healthy ulnar nerves. Among these sampled axons, 26 were accessible for NCV, 18 were successful for the strength-duration time constant, and 14 for both. Figure 1 shows an example of threshold tracking. Emergence of the sampled SMUP suggested the all-or-none law and was alternatively between ‘all’ and ‘none’ (Fig. 1A), as long as the test-pulse intensity (Fig. 1A) automatically adjusted along the conditioning-test interval (latent addition) (Fig. 1B) or the test-pulse duration (strength-duration curve) (Fig. 1C). Figure 2 exhibits the result of one latent addition test. Changes in the conditioned threshold are displayed as the percentage of the control threshold. The configuration of this result is similar to that demonstrated in previous reports (Bostock, Rothwell, 1997), although the curves are slightly uneven due to that the threshold tracking for a single axon is more difficult.
Asymmetry of the threshold change between depolarizing and hyperpolarizing conditionings was calculated by a simple subtraction of hyperpolarizing curves from depolarizing ones (Fig. 2, bottom). Because that the effect of passive cable property, which should affects the depolarizing and the hyperpolarizing sides in a same degree, has already been eliminated in the subtraction, the eventual curve reveals the active response of axonal membranes to a sub-threshold depolarizing stimulus. Waveform of this response is similar to that of the “local response”, showing to be transient and voltage-dependent. Little response has been elicited by the 30% conditioning pulse, i.e. axons presenting a large AUC value have a low threshold, and vice versa. This correlation is attributable to two explanations: 1) a geometric distribution of the sampled axons (e.g. the depth) that affects the unconditioned threshold also influences the AUC values, 2) fibers having a high AUC (a large, active response to the depolarizing pulse) are more excitable to the electric stimulation and, therefore, carry a lower threshold than the low AUC ones. Either of explanations, or both, can be right. The AUC in 26 NCV-available axons shows significantly positive correlation (p<0.005) to the NCV (Fig. 3B), which is not affected by the geometric factor, suggesting that the second explanation is however applicable.

Further analyses on the waveform reveal that the half-peak duration correlates with the threshold (Fig. 3C) and the NCV (Fig. 3D) as that for the AUC, while the peak height does not (Fig. 3E, 3F). In fact, the AUC itself is highly significantly correlated with the half-peak duration (p<0.000001) while uncorrelated with the peak height (p=0.114). This finding suggests that the...
Overall amount of the active sub-threshold response is mainly determined by the duration instead of the instant intensity of the response.

For the strength-duration study, AUC is inversely correlated to the rheobase (p<0.01). However, the correlation between the AUC and the SD time constant is not significant (p=0.401). From the estimated SD curve, the expected charge of threshold that was given to a single axon at the pulse duration of respective chronaxie, i.e., chronaxie (in msec) times expected current threshold (in mA) at chronaxie, was calculated. This charge threshold was, same as that for the rheobase, inversely correlated to the AUC (p<0.005). This result further confirmed that the amount of AUC was physiologically affected, and verified that the AUC represented the degree of local response on the axonal membrane, which reflect the subthreshold depolarizing sodium current.

**Multiunit preparation**

Two of the ulnar nerves in single unit study received surface CMAP tracking, for which the tracking target was set at the amplitude of CMAP that was elicited by the average current of control threshold of all sampled single units. In the single unit study, the waveform of the LR curves for each respected unit was not identical. They showed variation on the peak amplitude and the width. Their AUC were also highly varied. Despite these variation, the average waveform of single units was very similar to the results of surface multiunit tracking on the respective nerve (Fig. 4). This finding suggested that the surface recording, although in a multiunit preparation, could be used for LA studies, of which their results represented the average local responses of axons in the sampled nerve.

**Aminal studies**

AUC of rats before and after the injection of lidocain showed a good reproducibility before the injection (Fig. 5), and was uniformly decreased after the injection. This effect of lidocain lasted for about one hour. Because that the lidocain is a known sodium-channel blocker, this finding verifies our hypothesis that the AUC reflects the function of axonal sodium channels.
計劃成果自評：

本研究依照原來的計畫進行，最後的結果證明“潛時追加”檢查，確實可以直接用在人體身上來偵測神經軸突上鈉離子通道的功能。這個研究成果開啟了一扇窗戶，對於以後研究週邊神經功能及疾病上，有很大的幫助。

參考文獻：


