Sympathetic skin response evoked by laser skin stimulation

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Summary

The objective of this study was to evoke sympathetic skin responses (SSRs) in healthy subjects using laser stimulation and to compare these responses with those induced by conventional electrical stimuli. Twenty healthy subjects were investigated. SSRs were obtained using electrical and laser stimuli delivered to the wrist contralateral to the recording site. The sympathetic sudomotor conduction velocity (SSFCV) was measured in 8 subjects by simultaneously recording the SSR from the hand and the axilla.

The latency (L) of the laser-induced SSR (lSSR) was significantly longer than that of the electrically-evoked SSR (eSSR) (mean lSSRL = 1.7±0.14 ms, mean eSSRL = 1.56±0.14 ms, p<0.05). The amplitude (A) of the lSSR was lower than the eSSR amplitude (mean lSSRA = 1.31±0.26 mV, mean eSSRA = 2.59±0.49 mV, p<0.05). No significant difference between the lSSR and eSSR was observed in either the SSFCV or the variability and reproducibility parameters.

Our findings show that SSRs can easily be induced by laser stimuli and that this method shares the technical limitations of conventional eSSRs.

KEY WORDS: Laser stimulation, sudomotor fibres, sympathetic skin response.

Introduction

The sympathetic skin response (SSR) is a polysynaptic reflex generated in the deep layers of the skin by activation of sweat glands via sudomotor sympathetic efferent fibres (1). The SSR represents an unspecific arousal response to several endogenous (e.g., cough, inspiratory gasp) and exogenous (e.g., auditory, electrical or magnetic) stimuli (2). In clinical studies, the method most widely used to evoke SSRs is electrical stimulation. When using this technique the afferent pathway of the SSR is conveyed by large-myelinated somatosensory fibres. Although the central processing of the response is not fully understood, it seems to be influenced by input from various central nervous system structures such as the posterior hypothalamus and upper brain stem reticular formation (3).

CO₂ laser pulses selectively excite A-delta and C mechano-thermal nociceptors in the superficial skin layers (4). These highly selective nociceptive stimuli have been increasingly used to assess the functional integrity of small-fibre pathways in both pathophysiological and clinical settings (5,6).

The aim of this study was to evoke SSRs in healthy subjects using laser stimulation and to compare these responses with those induced by conventional electrical stimuli.

Materials and methods

Twenty normal volunteers (11 females and 9 males; age range 23-38 years; mean age 27.8±3.4 years; mean height 168±5.2 cm) gave their informed consent to participate in this study. Ethics Committee approval was obtained. No subjects had neurological diseases or were on medication affecting autonomic function.

Electrical and laser stimulation

Before performing the recordings the pain threshold of each subject was determined using the method of limits. The pain threshold was defined as the lowest intensity yielding a sensation described as “barely painful” on a standardised six-level verbal scale (7).

The electrically-induced sympathetic skin response (eSSR) was obtained using single square electrical pulses of 0.2 ms duration and 20-35 mA intensity, described as a “well perceived, non-painful” sensation, delivered to the wrist contralateral to the recording site. The laser-evoked SSR (lSSR) was obtained using a portable CO₂ laser stimulator (wavelength 10.6 μm, intensity 1.5-15 W, duration 15-20 ms, beam diameter 2.5 mm), (Neurolas, EL.EN., Florence Italy). The stimulus intensity (10-14 mJ/mm²) elicited a “barely painful” sensation in each subject. The laser beam was applied to the same stimulus site as the eSSR. The stimuli were delivered at irregular long intervals to avoid habituation, with an interval of >30s between stimuli. Laser and electrical stimuli were delivered in random succes-
session, in a single recording session conducted at 5 p.m. in all subjects so as to avoid circadian variations in SSRs (8). Two examinations, separated by an interval of 1-4 weeks were performed on 10 subjects to assess the reproducibility of the eSSRs and ISSRs.

**SSR recording technique and measurements**

SSRs were recorded in a quiet, brightly-lit room with the ambient temperature maintained at 26°C. The subjects were instructed to avoid abrupt limb movements, blinking, deep breathing and coughing. A headphone provided acoustic isolation. Care was taken to ensure that the subject remained awake and relaxed during the procedure.

SSRs were recorded using pairs of surface non-polarisable Ag/AgCl disk electrodes, applied to the palm (active electrode) and dorsum (reference electrode) of the hands. Signals were amplified, filtered (0.5-3000Hz) and stored by means of biopotential analysers (Premiere, Medelec, UK).

Owing to the correlation between skin temperature and SSR (9), a constant palm skin temperature of 32±0.5°C was maintained, if necessary by means of an infrared thermometer lamp.

To evaluate the efferent sympathetic pathways more selectively, in 8 subjects SSRs were recorded simultaneously in the same limb, both from the hand and the axilla (for this measurement the active electrode was placed on the axilla and referred to the positive one on the shoulder).

The response was considered absent if no consistent voltage change was seen when using a sensitivity of 50 μV per division after six or more trials. Only traces in which the onset latency was clearly detectable from the baseline were used for the calculation of the parameters. Eight responses were analysed for each subject after both electrical and laser stimulation. In order to render the method as accurate as possible, we calculated individual responses off-line. The occurrence rate (OR) of the eSSR and ISSR was calculated by considering the number of stimuli required to obtain eight responses (OR = 8/number of stimuli %). To minimise the effects of central processing on the SSR, only subjects presenting an OR > 65% were included in the statistical analysis because such subjects are believed to present a greater central facilitation and more stable responses (3).

The SSR latency (L) was defined as the time interval between the start of the stimulus and the first deflection of the signal from baseline. Amplitude (A) was measured from peak-to-peak of the response.

We used three different latency and amplitude measurements: mean latency and amplitude (Lm, Am: mean values of the eight responses); average latency and amplitude (Lavg, Aavg: values obtained from the averaged SSRs); median latency and amplitude (Lmd, Amd).

We analysed the intra-individual variability of ISSR and eSSR parameters by using a variation coefficient (CV = SD/mean x 100).

The reproducibility of the ISSR and eSSR was calculated by means of the latency variability rate (VR) (latency difference between the first and second test / latency values in the first test x 100).

The sudomotor sympathetic fibre conduction velocity (SSFCV) was measured for both the ISSR and eSSR by dividing the distance between the proximal and distal recording sites by the difference between the latencies of the two corresponding responses.

**Data analysis**

Statistical evaluation included calculation of means±standard deviations and the paired t-test. p<0.05 was taken as the level of significance.

**Results**

**Shape, latency and amplitude of responses**

SSRs were present in all the subjects after both electrical and laser stimulation. Four subjects in whom the OR was lower than 65% were excluded from the statistical analysis, in accordance with our inclusion criteria. Figure 1 illustrates the sudomotor potential following

![Fig. 1 - Sympathetic skin response evoked by electrical (eSSR) and laser (ISSR) stimulation in a representative subject at proximal (axilla) and distal (hand) recording site (average of 8 trials). Horizontal calibration: 500 ms. Vertical calibration: 500 μV.](image-url)
laser and electrical stimulation. The shapes of the response were similar after both types of stimulation in most of the subjects and were mainly biphasic with a predominant positive component in 62% of the subjects. The eSSR anticipated the lSSR in all the subjects and the ISSRL was significantly shorter than the eSSRL for every parameter (Table I). The mean difference between the ISSRL and eSSRL ranged between 120 and 140 ms. No significant difference was found between the mean intra-individual CV of the eSSR (5.3±0.84) and ISSR (6.97±0.58, paired t-test, p>0.05).

Amplitude varied from test to test despite efforts to limit habituation. The mean amplitudes of the ISSRs were significantly lower than those of the eSSRs (Table I). No significant difference was found between the CVs of the two responses (eSSR 29.5±7.2; ISSR 26.2±6.7, paired t-test, p>0.05).

SSFCV

A clear SSR was obtained from both the proximal and the distal sites in all the subjects (Fig. 1). The conduction velocity was 2.07±0.55 m/s and 2.12±0.61 m/s for the lSSR and eSSR respectively (paired t-test, p>0.05).

Reproducibility of ISSR and eSSR

No significant changes in latency were noted following either stimulation type, using the paired t-test for comparison of means, in the ten patients who underwent repeat testing after 1-4 weeks. No significant differences were found, between the eSSR and ISSR, in the latency variability rates of the parameters considered (Table I).

Discussion

In the present study we demonstrate that SSRs can easily be obtained in response to laser stimulation of human upper limbs. Laser stimulation was as effective as electrical stimulation in eliciting SSRs. In the ISSRs, the onset latency was constantly longer and the amplitude smaller than in the eSSRs. The mean difference between eSSR and ISSR latency was approximately 130 ms, with minor differences between the different measurements (Lavg, Lm, Lmd).

The delay in the ISSRL may be partially explained by the fact that the afferent side of the reflex arc in the ISSR is different from that in the eSSR, while electrical stimuli are in fact thought to be conveyed by large myelinated sensory fibres conducting at 40-60 m/s (3), the peripheral afferent volleys of the ISSR are conducted by small myelinated (A-delta) primary sensory neurons (10). Previous studies have estimated the conduc-

Table I - Laser and electrically evoked palmar sympathetic skin response: measurements obtained in 16 healthy volunteers. (Eight responses per subject were analysed).

<table>
<thead>
<tr>
<th></th>
<th>ISSR</th>
<th>eSSR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Lm (s)</td>
<td>1.7 ± 0.145</td>
<td>1.56 ± 0.14*</td>
</tr>
<tr>
<td></td>
<td>1.36 – 2.14</td>
<td>1.19 – 2.02</td>
</tr>
<tr>
<td>VR (%)</td>
<td>10.2 ± 4.7</td>
<td>8.8 ± 4.8</td>
</tr>
<tr>
<td>Lavg (s)</td>
<td>1.68 ± 0.174</td>
<td>1.56 ± 0.144*</td>
</tr>
<tr>
<td></td>
<td>1.28 – 2.04</td>
<td>1.18 – 2.02</td>
</tr>
<tr>
<td>VR (%)</td>
<td>12.9 ± 5.1</td>
<td>9.4 ± 4.8</td>
</tr>
<tr>
<td>Lmd (s)</td>
<td>1.7 ± 0.146</td>
<td>1.57 ± 0.13*</td>
</tr>
<tr>
<td></td>
<td>1.34 – 2.1</td>
<td>1.18 – 1.96</td>
</tr>
<tr>
<td>VR (%)</td>
<td>9.1 ± 5</td>
<td>6.7 ± 4.3</td>
</tr>
<tr>
<td>Am (μV)</td>
<td>1.31 ± 0.26</td>
<td>2.59 ± 0.49*</td>
</tr>
<tr>
<td></td>
<td>0.9 – 1.8</td>
<td>1.9 – 4.15</td>
</tr>
<tr>
<td>Aavg (μV)</td>
<td>1.23 ± 0.26</td>
<td>2.57 ± 0.61*</td>
</tr>
<tr>
<td></td>
<td>0.8 – 1.7</td>
<td>2 – 4.8</td>
</tr>
<tr>
<td>Amd (μV)</td>
<td>1.28 ± 0.27</td>
<td>2.53 ± 0.52*</td>
</tr>
<tr>
<td></td>
<td>0.9 – 1.68</td>
<td>2.1 – 4.32</td>
</tr>
</tbody>
</table>

Abbreviations: ISSR = laser evoked sympathetic skin response; eSSR = electrically evoked sympathetic skin response; Lm, Am, Lavg, Aavg, Lmd, Amd: latency and amplitude measurements calculated as described in the Methods; VR: variability rate calculated as described in the Methods.

*Significantly different from ISSR, p<0.05.
tion velocity of A-delta fibres after the application of laser stimuli to be 9-14 m/s (11). By assuming a mean distance value of 80 cm between the stimulus site and the spinal cord entry level (C7 vertebra), the peripheral afferent time of the eSSR and ISSR may be estimated to be 15 ms and 65 ms, respectively.

The nociceptor activation time of the laser beam has been estimated to be 40 ms for an A-delta fibre nociceptor (12). The different peripheral afferent pathways may consequently account for a delay of approximately 90 ms for the ISSR. Thus, the observed mean differences between the ISSR and eSSR exceeded by 40 ms the expected value calculated on the basis of the different afferent time of the reflex arc.

The efferent side of the reflex pathway, on the other hand, is believed to be the same in the eSSR and in the ISSR given the similarity of the SSFCV values calculated in the axilla-hand tract. In this regard, it may be speculated that different central polysynaptic pathways and/or processing times may be involved in the production of the SSR evoked by laser and electrical stimulation.

The fact that the variability and reproducibility of the ISSR were similar to those of the eSSR indicates that the technical factors adversely affecting the accuracy of SSR measurement are similar after both types of stimulation. The amplitude of the ISSR, also observed when other kinds of stimuli are used (13,14), varied greatly and hence precludes its use as a reliable measure of the SSR.

In conclusion, our findings show that SSRs can easily be induced by laser stimuli; this method, however, shares the same technical limitations as conventional eSSRs. Laser stimulation allows a selective activation of small-fibre afferents. A combination of SSR stimulus types (e.g., auditory, electrical or magnetic coupled with laser stimuli) could help to differentiate between afferent small and efferent sympathetic nerve fibre dysfunction.

References