Pain fibers contribute to the generation of subcortical somatosensory evoked potentials (SEPs) in rats

Fuad A. Abdulla
Department of Physical Therapy, Faculty of Allied Health Sciences, Hashemite University, Zarqa, Jordan
Reprint requests to: Dr Fuad A. Abdulla
Department of Physical Therapy
Faculty of Allied Health Sciences
The Hashemite University, P.O. Box 150459, 13115 Zarqa, Jordan
E-mail: fabdulla@hu.edu.jo
Accepted for publication: February 9, 2005

Summary
Subcortical somatosensory evoked potentials (SEPs) were obtained by electrical stimulation of the volar surface of the forepaw and were recorded from the skull overlying the contralateral somatosensory area of the cerebral cortex. Three distinct peaks were discernable in the SEPs prior to the first cortical component. Dorsal column transection at C3 level reduced the amplitude of component III by 48.7±4.7% without affecting the amplitude of components I and II. Morphine given either i.v. (2.5 mg/kg) or intrathalamically (25 \( \mu \)g/kg) almost completely abolished the part of component III that remained after surgical sectioning of the dorsal column of the spinal cord. The effects of morphine were reversed by i.v. naloxone (0.25 mg/kg). These results indicate that both the dorsal column and the anterolateral systems contribute significantly to the generation of component III of subcortical SEPs. Subcortical SEPs may be a useful non-invasive technique for studying the neurophysiological effects of known and experimental analgesics.

KEY WORDS: dorsal column transection, dorsal rhizotomy, far-field subcortical somatosensory evoked potentials, morphine, pain.

Introduction
Far-field somatosensory evoked potentials consist of three distinct subcortical peaks that originate within the neuraxis. There may be additional wavelets on the peaks (1,2). The genesis of the first peak is attributed to the spinal cord. The second peak arises from the brain stem dorsal column nuclei and the medial lemniscus. The third peak originates from the thalamus and the thalamic radiation (1,2). There are two main ascending systems for somatic sensation: the dorsal column-medial lemniscal system, which mediates tactile sensation and proprioception, and the anterolateral system, which mediates pain and temperature sensation (3). Results from previous work in our laboratory suggested that small diameter fibers may contribute to the generation of subcortical somatosensory evoked potentials (SEPs) recorded in response to electrical stimulation (4,5). Clinically, SEPs are usually recorded in response to electrical stimulation. The aim of the present study was to identify putatively extralemniscal far-field potentials a) through their association with activity of high threshold primary afferents, b) through elimination of lemniscal input, by dorsal column transection, and c) through their selective modulation with opiate and opiate antagonist. Thus, we tested the hypothesis that small diameter fibers contribute significantly to the generation of subcortical SEPs. In the past three decades several research laboratories have been engaged in the difficult task of developing an objective method for studying the physiological functions of small diameter fibers mediating pain-temperature sensation (6,7). The results of the present study may contribute to this debate. Conduction studies using electrical stimulation are non-invasive, inexpensive and simple to perform, and they may prove to be important in the study of pain in general and provide a useful model for investigating the neurophysiological effects of known and potential analgesics.

Materials and Methods
Subjects
Experiments were performed according to protocols approved by the Institutional Animal Care Committee of the Hashemite University. Male Wistar rats (locally bred) weighing between 270-350 g (320±31 g) were used. The animals were housed 3-4 per cage, with free access to food and water under an alternating 12 h light and dark cycle at 23±1°C.

Procedure
The rats were anesthetized with 50 mg/kg pentobarbitone sodium (i.p., May and Baker, Dagenham, UK). Heart rate was monitored using a chest lead ECG system and a steady level of anesthesia was maintained, keeping the heart rate at 400±20 beats/minute. Rectal temperature was maintained at 37.0±0.2°C. The femoral vein was cannulated for drug injection. Each animal was fixed in a stereotaxic frame (David Kopf Instruments, California) as described by Pellegri-no et al. (8). The skull was exposed and a small stainless steel screw (1.0 mm in diameter) was implanted 1.0 mm anterior and 3.0 mm lateral to the bregma to serve as a recording electrode. Another screw was
implanted in the nasal bone 20 mm anterior to the bregma to serve as a reference electrode. Leads from a digital oscilloscope (Pathfinder II, Nicolet Instruments Corp., Madison, WI) were attached to the exposed heads of the screws. Evoked potentials were obtained on electrical stimulation of the contralateral forepaw (1.8±0.2 mA, 100 μsec, 1.7 Hz). Further details of the techniques used were published in an earlier study (4).

Compound action potentials (CAPs) were also recorded from the median nerve. The median nerve was exposed and placed on stainless steel hook electrodes. The nerve in between the recording electrodes was crushed to record a monophasic CAP. The recording was obtained using filter settings: low band pass of 0.1 Hz and high band pass of 3000 Hz. The latencies of peripheral CAPs and the distances between the stimulating and the recording electrodes were used to calculate the conduction velocity of different fiber groups. Stimulus strength, duration and frequency were the same as those used for recording far-field subcortical SEPs.

Surgical sectioning of the dorsal column of the spinal cord

Surgery was performed under 50 mg/kg pentobarbital sodium anesthesia. The skin of the cervical region was shaved. A midline incision was made from the occiput to the 7th cervical vertebra. Norepinephrine (l:50,000) was infiltrated into the neck muscle to cause local vasoconstriction. The vertebral column was covered with a cotton swab soaked in normal saline at 37°C. A scouten adjustable wire knife (model 120, David Kopf Instruments, California) was attached to the exposed heads of the screws. Evoked potentials were recorded heads of the screws. Evoked potentials were recorded from the median nerve. The median nerve was exposed and placed on stainless steel hook electrodes. The nerve in between the recording electrodes was crushed to record a monophasic CAP. The recording was obtained using filter settings: low band pass of 0.1 Hz and high band pass of 3000 Hz. The latencies of peripheral CAPs and the distances between the stimulating and the recording electrodes were used to calculate the conduction velocity of different fiber groups. Stimulus strength, duration and frequency were the same as those used for recording far-field subcortical SEPs.

Effects of morphine on subcortical SEPs were studied. Morphine was administered intravenously (2.5 mg/kg), the dose at which maximum effect on subcortical SEPs was obtained (4.5), or intrathalamically (25 μg/kg). Intrathalamic infusion of morphine was achieved through an injection cannula (gauge 27), inserted through a stereotaxically implanted guide cannula (gauge 21, A=3.5 mm, L=2.0 mm and V=3.0 mm). The tip of the injection cannula protruded 3 mm beyond that of the guide cannula. To check for any non-specific effects of the injection, an equal volume of vehicle (sterile saline) was similarly infused. The locations of the microinfusion cannulae were confirmed by examining 35 μm thick histological sections. Reversibility of morphine effect was studied by injecting the morphine antagonist, naloxone (0.25 mg/kg, i.v., naloxone hydrochloride, Sigma, Saint Louis).

Data analysis

Peak-to-peak amplitudes of the three subcortical components of the SEPs were analyzed. Paired t-test and Student’s t-test were used to assess the drug’s effects. Paired t-test was used to assess DCT effects. The differences were considered significant at p<0.05. All data are expressed as mean values ± S.E., unless otherwise indicated.

Results

Median nerve compound action potential

The median nerve CAP consisted of two peaks (Fig. 1). The threshold of the first peak was always lower than the threshold of the second peak, indicating that two distinct groups of nerve fibers contributed to these peaks (9). The amplitude of both peaks increased in a graded manner as the strength of stimulation was increased. No significant change in the amplitudes of the two peaks was observed when the stimulus strength was increased above 1.8 mA. The conduction velocity for points X (the beginning of the response), Y and Z (the first and second peak, respectively, see Fig. 1) were 47.4±2.4 m/sec, 28.1±1.94 m/sec and 19.2±0.91 m/sec, respectively (n=6 rats), indicating that both Aδ and Aβ fibers contribute to the median nerve CAP (9).

Subcortical SEPs

Subcortical SEPs consist of three distinct peaks prior to the cortical component (Fig. 2). Figure 2 shows that above threshold stimulus strength, the amplitude of the three subcortical components increased in a graded manner as the stimulus strength was increased. Components I and II reached maximum amplitude at 1.2 mA (i.e., 4 times the threshold stimulus strength, whereas component III reached its maximum amplitude at stimulus strength of 1.8 mA (i.e., 6 times...
the threshold stimulus strength of 0.3 mA). These observations indicate that recruitment occurs between threshold stimulus strength and four times the threshold stimulus strength. In the case of components I and II, the recruitment phenomena are complete at four times the threshold stimulus strength, whereas recruitment of component III continues and reaches its maximum at 6 times the threshold stimulus strength. On further increasing the stimulus strength (i.e., above 6 times the threshold) the amplitudes of the three subcortical SEP components did not increase significantly. Therefore, stimulus strength 6 times the threshold was used to evoke the subcortical SEPs. The mean stimulus threshold from 8 rats was 0.3±0.06 mA.

Effects of dorsal column transection (DCT)

In three rats, laminectomy alone reduced the amplitude of component III by 20-30%, therefore these rats were excluded. In the rest of the animals (n=10 rats) laminectomy did not cause any significant effects on the amplitude of the three subcortical SEP components. Dorsal column transection at C3 level significantly reduced the amplitude of component III (by 48.7±4.3%; p<0.001), without affecting the amplitude of the other two components (Fig. 3, A,B, over). The onset and the peak latencies of the three SEP components were prolonged but did not reach statistical significance.

In two experiments, DCT at the midthoracic level produced no effects on the amplitudes or latencies of the three SEP components. Dorsal column transection reduced the amplitude of component III by eliminating (or at least reducing) the contribution of large diameter fibers, therefore it may be expected that if only the large fibers were stimulated, DCT would completely eliminate component III. In three experiments, DCT completely eliminated component III when evoked using stimulus strength 3 times the threshold (3 times the threshold corresponds to the threshold of the second peak of the median nerve CAP, see Fig. 1). The amplitude of the other two components was unaffected (Fig. 4, over).

To investigate the nature of fiber types contributing to the generation of what remains of component III and of components I and II, we studied the effects of morphine on the responses persisting after DCT. Five min after i.v. morphine injection (2.5 mg/kg, n=5 rats) the remaining part of component III was almost completely abolished, without affecting the amplitudes of the other two components (Fig. 3, over). The onset and peak latencies of the three components were not significantly affected by morphine injection. Similarly, direct microinfusion of morphine (25 µg/kg, n=5 rats) into the thalamus (the origin of component III) almost completely abolished those parts...
of component III that remained after DCT without affecting the amplitude of the other two components or the latencies of the three components (Fig. 5). Intravenous naloxone (0.25 mg/kg) completely reversed the effects of morphine given intravenously and intrathalamically. In fact, the amplitude of component III in the post-naloxone recording was greater than that in the pre-morphine recording (Fig. 3D, Fig. 5D) (4).

In an attempt to understand why components I and II were not affected by DCT or by morphine, the effects of C2-T1 dorsal rhizotomy were studied in three rats. This lesion resulted in complete abolition of components II and III without significantly affecting component I (Fig. 6).

**Histological confirmation**

Spinal cord sections were analyzed under a microscope to confirm the extent of the lesion. The sections showed marginal differences in the areas damaged by the lesion. In all the sections studied, common damaged areas included: the ipsilateral dorsal gray horn, the ipsilateral dorsal white column, the dorsal part of the lateral white column, and the contralateral fascicules gracilis (Fig. 7). In two sections the contralateral cuneate fascicules was also damaged. However, in all sections the ventral gray horn and the anterolateral system were completely spared. The tracts and the tips of the injection cannulae were identified in the thalamic sections. Three tips were located in the thalamic radiation, one in the central nucleus of the thalamus and one in the ventrobasal complex.
Discussion

Unilateral DCT at C3 level completely destroyed the dorsal column input (mainly large diameter fibers) from the ipsilateral forepaw to the contralateral thalamus. However, the small diameter afferent input to the thalamus via the anterolateral system and other spinal ascending pathways to the cerebellum and the brain stem structures remained intact. In this preparation, the amplitude of component III was reduced by about 49%, whereas the amplitude of the other two components was unaffected. Reduction in the amplitude of component III after DCT suggested that it is partly originated from dorsal column pathways (other pathways may contribute to the generation of this component), in agreement with the finding of Wiederholt and Iragui-Madoz (1).

About 51% of component III remained intact in a DCT preparation indicating that other pathways, including the transmission of impulses through the anterolateral system, were also required for the development of this wave. Morphine (intravenously or intrathalamically administered) almost completely abolished the remaining part of component III in a DCT preparation. In fact, i.v. morphine given to intact rats reduced the amplitude of component III by 46% (4,5). Since morphine selectively blocks small diameter fibers (10-12) this observation supports our hypothesis that impulses ascending via the anterolateral system contribute significantly to the generation of component III. These findings also suggest that the contribution of other ascending pathways, (e.g., the spinocerebellar system) to component III was insignificant.

In DCT preparations a prolongation of the latency of component III was expected given that, following DCT, impulses to the thalamus are transmitted mainly through the small diameter fibers. However, the observed increase in peak latency of component III was statistically non-significant. A partial explanation for this may be that the majority of the fibers that contributed to the peak of wave III were small in diameter. One may also consider the evidence that spinal pathways carrying small fiber activities in a rostral direction were faster than originally thought. Conduction velocity greater than 35 m/sec has been reported (13). Therefore, it is possible that slowly conducted input to the spinal cord might project by fast-conducting pathways to the thalamus, which would explain why the prolongation in the latencies was not matched by the decreased conduction velocity expected in a DCT preparation. However, this explanation is not supported by the finding that conduction velocity of the central tracts is similar to or slightly slower than the conduction velocity of their peripheral pathways (14). Alternatively, a faster central conduction velocity could be due to disinhibition from descending pathways, such as the coeruleospinal and raphe-spinal pathways (15,16). Other explanations cannot be excluded.

Component II was completely abolished following C2-T1 dorsal rhizotomy, indicating that this component originated central to the lesion site. However, neither DCT nor morphine infusion affected the amplitude of component II. There are various possible explanations for the finding that DCT did not affect component II. Maybe this wave, recorded after DCT, originated from the dorsal column below the site of the lesion; the finding may also (or alternatively) be explained by activities of those ascending tracts that remained intact in the DCT preparation. Component I persisted after rhizotomy and was unaffected by DCT. This conclusion is in agreement with the finding of Allison and Hume (17), who reported that waves N12a and N12b, which had mean peak latencies of 1.7 and 2.5 msec, respectively — the peak latency of component I in the present experiment was 1.98±0.2 msec —, originate from primary afferent fibers.

The absence of an effect of morphine on components I and II could be due to the lack of opiate receptors in the...
generators and pathways of these subcortical components of SEPs. Maurer et al. (18) reported that there were very few opioid receptors in the cuneate nucleus; see also Mansour and Watson (19).

In conclusion, the findings of the present study showed that both the dorsal column and the anterolateral systems contribute to the generation of subcortical SEPs. They also indicate that subcortical SEPs may be a useful non-invasive technique for studying the neurophysiological effects of known and experimental analgesics.

Acknowledgments

This study was supported in part by the Hashemite University. I would like to thank Dr Esam Y. Qnais for commenting on the original manuscript.

References

5. Abdulla FA, Aneja IS. Morphine inhibits the thalamic component of the subcortical somatosensory evoked potentials in rats. Funct Neurol 1993;8:197-203
16. Sandkuhler J, Gebhart GF. Relative contributions of the nucleus raphe magnus and adjacent medullary reticular formation to the inhibition by stimulation in the peri-aque ductal gray of a spinal nociceptive reflex in the pentobarbital-anesthetized rat. Brain Res 1984;305:77-87