Further Evidence for Possible Analgesic Mechanism of Electroacupuncture: Effects on Neuropeptides and Serotonergic Neurons in Rat Spinal Cord

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Abstract—The possible mechanism of electroacupuncture (EAc) in reference to the effects of neuropeptides on serotonergic neurons in rat spinal cord was studied. The tested drugs were administered by intrathecal injection or spinal push-pull perfusion. The results showed that baclofen, substance P (SP) and naloxone administered intrathecally could reduce the tail pressure pain threshold. The pain threshold was increased by met-enkephalin (EK) and EAc. The action of EAc was antagonized by naloxone. The release of 5-HT in the spinal cord evoked by tail pressure pain stimulation (TP) was inhibited by EK, baclofen and EAc. However, naloxone could potentiate the 5-HT release evoked by TP. EAc reversed the naloxone potentiation of TP-evoked 5-HT release. The 5-HT release evoked by exogenous SP, however, was potentiated by EK and EAc. From these results, it is suggested that the influence of EAc on 5-HT release may be due to activation of enkephalin-interneurons, which presynaptically inhibit the primary sensory neurons in the spinal cord.

Many studies have shown that descending serotonergic fibers from the raphe nuclei are associated with pain regulation (1, 2), and that the ascending serotonergic fiber play an important role in acupuncture analgesia (3, 4). Cheng and Pomeranz (5) suggested that electroacupuncture analgesia induced by low frequency stimulation may be mediated by endorphins, while high frequency stimulation is not endorphinergic but may be partly due to serotonin. In our previous studies, it has been shown that 5-HT release from a specimen of spinal dorsal horn was significantly stimulated by somatostatin and substance P in vitro, but not by neurotensin and met-enkephalin (6).

Moreover, the substance P-stimulated 5-HT release was strongly inhibited by GABA and baclofen, but not by met-enkephalin in rat spinal slices (6). In intact rats, the 5-HT release from the spinal cord could be evoked by substance P and tail pinch, but not by met-enkephalin. The substance P-evoked 5-HT release was slightly potentiated by met-enkephalin (7).

These findings have shown that the descending serotonergic neurons are modulated by neuropeptides of interneurons in the spinal cord. In the present study, we further explore possible mechanism of antinociception in electroacupuncture on neuropeptide and serotonergic neurons in rat spinal cord.

Materials and Methods

Electroacupuncture and intrathecal administration of various drugs in spinal operated rats: Sprague-Dawley rats weighing between 150 and 200 g were anesthetized with ether. A polyethylene tube (No. 10), used as an intrathecal cannula (ca. 20 cm), was inserted into the spinal subarachnoid space (ca. 1.5 cm) from the lumbosacral enlargement (5). The rat was then placed in a rat holder. The test was begun 3 hr after the
initial operation (at this time, the pain threshold was recovered to the pre-operative level). The pain threshold was determined as tail withdrawal and/or an attempt to turn the head in reaction to the pressure pain stimuli by a pressure analgesy meter (Model MK-300, Muromachi Kikai Co.). The pain thresholds were measured at 5, 20, 35, 65 and 95 min after drug administration. Electroacupuncture was given by inserting fine stainless acupuncture needles at the points analogous to the traditional Chinese acupuncture literature Tsu-San-Li (S 36) in both hind feet. Electroacupuncture was applied for 5 min by means of square pulses from a stimulator at 2 Hz, 0.5 msec and 3–5 V. The voltages were adjusted to be just above threshold for muscle twitching. Five min after stimulation, the pain thresholds were measured at 5, 20, 35, 65 and 95 min.

Drugs (40 μl) employed: met-enkephalin (40 μM), baclofen (40 μM), substance P (40 μM) and artificial CSF (control group) were administered from intrathecal cannula. The artificial CSF is an oxygenated Krebs-bicarbonate solution (compositions: 120 mM NaCl, 5 mM KCl, 15 mM NaHCO3, 1.0 mM MgSO4, 1.5 mM CaCl2 and 10 mM glucose).

Measurement of 3H-5-HT release from rat spinal cord by spinal push-pull perfusion: The experiment was performed by spinal push-pull perfusion in vivo as described in our previous paper (7). Male Sprague-Dawley rats, weighing between 200 and 250 g, were anesthetized with urethane (1.2 g/kg, i.p.). The stainless-steel guide cannula as a pull-tube was inserted into the cisterna magna and a polyethylene tube (No. 10) as push-tube was inserted into the spinal subarachnoid space (ca. 1.5 cm) from the lumbo-sacral enlargement. The perfusion medium was the oxygenated artificial CSF solution described above, which contained 7.5 × 10^{-5} M pargyline to prevent the metabolism of 3H-5-HT.

First, the spinal cord was incubated by perfusion oxygenated artificial CSF solution containing 3H-5-HT (1 μCi specific activity: 32.1 Ci/mnmole) for 40 min at 37°C. After the incubation, the spinal cord was continuously perfused with oxygenated artificial CSF solution containing 7.5 × 10^{-5} M desipramine for 20 min. Various neuropeptides and other substances (e.g., baclofen) were added into the medium by 15 min applications. The perfused samples were collected at 5 min intervals (at a rate of 0.25 ml/min) through the pull-tube.

Pain was produced by pinching the rat tail with a constant pressure (ca. 600 g) for 5 min. Electroacupuncture was given for 15 min by passing square pulses from a stimulator at 5V, 0.5 msec and 10 Hz at the points of Tsu-San-Li (S 36). The frequency was adjusted to 10 Hz to keep the rat from shaking during the push-pull perfusion. The radioactivity in each fraction was analyzed by a liquid scintillation spectrometer (Aloka LSC-673), and the release of 5-HT was expressed as dpm × 10^2/tube. The baseline for each sample taken after the stimulation was estimated by extrapolating the stable washout curve from the 6th preceding sample in a manner similar to that described by Yaksh and Yamamura (8).

Statistical analysis of differences in a paired experiment was performed using Student's t-test.

**Results**

Effect of electroacupuncture and intrathecal neuroactive peptides on the changes of pain threshold in spinal operated rats: The change of pain threshold due to the intrathecal administration of met-enkephalin (EK, 40 μM), baclofen (40 μM), and substance P (40 μM) and artificial CSF (control group) were administered from intrathecal cannula. The artificial CSF is an oxygenated Krebs-bicarbonate solution (compositions: 120 mM NaCl, 5 mM KCl, 15 mM NaHCO3, 1.0 mM MgSO4, 1.5 mM CaCl2 and 10 mM glucose).

Effect of electroacupuncture and neuroactive peptides on the 5-HT release evoked by tail pain stimulation: The change of pain threshold due to the intrathecal administration of met-enkephalin (EK, 40 μM), baclofen (40 μM), and substance P (SP, 40 μM) were observed in spinal operated rats (Fig. 1). The pain threshold was increased by EAc, while decreased by baclofen and substance P. As shown in Fig. 2, EAc increased the pain threshold, while naloxone decreased it. Increased pain threshold due to EAc was reversed by naloxone (P<0.001) to almost the level of naloxone only.

Effect of electroacupuncture and neuroactive peptides on the 5-HT release evoked by tail pain stimulation: The release of 5-HT from rat spinal cord could be markedly evoked by tail pain stimulation (TP). This 5-HT release evoked by TP was significantly inhibited by baclofen (40 μM), met-enkephalin (40 μM) and EAc (P<0.01) (Fig. 3). Naloxone (40 μM) could potentiate the TP-evoked
5-HT release (P<0.01) (Fig. 4). EAc reversed naloxone potentiation of TP-evoked 5-HT release. However, the 5-HT release evoked by exogenous substance P was potentiated by met-enkephalin and EAc (P<0.05) (Fig. 5).

Discussion

The descending serotonergic fibers that originate from the nucleus raphe, proceeding to the spinal dorsolateral funiculus, and terminating on the enkephalinergic neurons are associated with pain regulation (9-11).

It is, therefore, evident that the spinal serotoninergic system mediated the antinociceptive of morphine (12-14) and its excitation elevates the nociceptive threshold (15). A previous study suggested that the peripheral
pain stimulation-produced 5-HT release in the spinal cord might be mediated via an intrinsic substance P released from the primary sensory neurons (7). From the present experiment, it was observed that the 5-HT release evoked by exogenous substance P was enhanced by EAc and enkephalin. However, the 5-HT release induced by peripheral tail pain stimulation was inhibited by EAc and enkephalin. This conflicting result of EAc and enkephalin upon 5-HT release due to different stimulations may be reasonable if the following assumption is made: Enkephalin inhibits presynaptically the primary afferent nerve and exogenous enkephalin apparently acts in an inhibitory manner.

In the present study, substance P as well as baclofen lowered the pain threshold. There have been many papers dealing with baclofen analgesia (16-19). It was evident that baclofen analgesia within the spinal cord was not antagonized by bicuculline, but was via a special baclofen receptor rather than the GABA receptor (19).

Despite the inhibition of baclofen on 5-HT release both by tail pinch stimulation and substance P-stimulation in vitro (6), baclofen did not demonstrate analgesia, but rather showed algesia in the present experiment. Although further investigation is necessary for different doses of baclofen, we can speculate
that intrathecal baclofen might show strong inhibitory action on 5-HT release, in a manner different from the actions of enkephalin and EAc, because it was evident from this study that the site of action of baclofen was also different from those of enkephalin and EAc.

Since inhibition of EAc on 5-HT release and the analgesic action of EAc were both naloxone-reversible in this study, it was conclusive that the analgesic action of EAc in the spinal cord may be via the stimulation of enkephalin-interneurons and the influence of EAc on 5-HT release may be due to activation of enkephalin-interneurons in the spinal cord.

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References
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