A Possible Involvement of Monoaminergic and Opioidergic Systems in the Analgesia Induced by Electro-Acupuncture in Rabbits

Jun Takagi¹, Takanori Sawada¹ and Norifumi Yonehara²,*
Departments of ¹Anesthesiology and ²Pharmacology, Osaka University Faculty of Dentistry, 1-8 Yamadaoka, Suita, Osaka 565, Japan

ABSTRACT—To elucidate mechanisms involved in analgesia induced by effects of electro-acupuncture (EAP), effects of EAP on evoked potentials and release of substance P (SP) following tooth pulp stimulation (ST) in the superficial layers of the trigeminal nucleus caudalis (Vc-I–II) were studied in the rabbit. The potentials evoked by ST were composed of two main components with conduction velocity of ca. 30 m/sec (fast component) and ca. 12 m/sec (late component). The late component was significantly inhibited by morphine (10 mg/kg, i.v.) or CP-96,345 (5 mg/kg, i.v.), an SP antagonist. This inhibitory effect of morphine was antagonized by naloxone (1 mg/kg, i.v.) or methysergide (5 mg/kg, i.v.). In addition, the late component was significantly inhibited by EAP, which was observed in ca. 70% of the rabbits examined. This EAP-induced inhibitory effect was antagonized by naloxone (1 mg/kg, i.v.) or methysergide (5 mg/kg, i.v.), but not by prazosin (5 mg/kg, i.v.) and yohimbine (1 mg/kg, i.v.). The stimulus-evoked SP release was inhibited by EAP, which was significantly antagonized by pretreatment with naloxone (1 mg/kg, i.v.) or methysergide (5 mg/kg, i.v.). These results suggest that one of the mechanisms of analgesia induced by EAP is due to inhibition of the stimulus-evoked SP release in the Vc-I–II through activation of the ascending serotonergic systems linking up with opioidergic systems.

Keywords: Electro-acupuncture, Tooth pulp stimulation, Evoked potential, Trigeminal nucleus caudalis, Serotonin

The spinal trigeminal nucleus (STN) is involved in the perception and transmission of orofacial sensory information from the trigeminal, facial, glossopharyngeal and vagal nerves (1). The STN is divided into three subnuclei from rostral to caudal: oralis, interpolaris and caudalis (1, 2). Among these subnuclei, the subnucleus caudalis is considered to be functionally and anatomically analogous to the dorsal horn (3) and a site for the relay nucleus of the craniofacial nociceptive information to higher levels of the brain (4). Substance P (SP)-like immunoreactivity has been found in primary afferent fibers from the tooth pulp that terminate in the superficial layers of the subnucleus caudalis of STN (Vc-I–II) (5). Furthermore, iontophoretically applied SP facilitates activities of neurons located in the subnucleus caudalis that responded to noxious cutaneous stimuli and/or to electrical stimulation applied to the tooth pulp (6). These findings suggested that SP might play an important role in the transmission of pain in the trigeminal afferent nociceptive pathways.

We previously observed that electrical stimulation of the tooth pulp (ST) caused a significant increase in release of SP from the Vc-I–II (7). We also proposed based on a series of our studies that nociceptive transmission in the region of the Vc-I–II is modified by an intrinsic opioid system and a descending monoaminergic system, particularly, the descending 5-HT pathway (7–9).

There is considerable evidence that acupuncture, an ancient Chinese art of healing, is useful in some patients with pain (10, 11). Moreover, a rise of the tooth pain threshold in healthy volunteers has been observed during acupuncture treatment (12, 13). Concerning the mechanisms of analgesia induced by acupuncture, some studies have demonstrated that acupuncture can modulate nociceptive impulses in the central nervous system by activating certain somatosensory pathways including neurohumoral and opioid systems. In a recent study, we reported that electro-acupuncture (EAP) inhibits increased release of SP and amplitudes of field potentials induced by ST in the Vc-I–II (14).

In the present study, therefore, to elucidate whether the
descending monoaminergic systems are involved in the mechanism of the effect of EAP, we examined the effects of compounds related to monoamine on the inhibitory effect of EAP to stimulus-evoked SP release and potential in the Vc-I–II.

MATERIALS AND METHODS

Recording of evoked potential and perfusion of the Vc-I–II

Male rabbits, weighing 2.5–3.5 kg (Nihon-Dobutsu, Osaka), were anesthetized with urethane (1 g/kg, i.p.) and artificially ventilated. Blood pressure and rectal temperature were continuously monitored, and respiratory parameters were adjusted to keep pCO2 near 35 mmHg. A bipolar, enamel-insulated stimulating electrode (0.1-mm diameter, 0.5-mm exposed tip) was inserted into the pulp of the lower incisor and fixed with dental cement and resin. The animal was then placed in a stereotaxic apparatus, and then a push-pull cannula for perfusion experiment or a monopolar recording stainless steel electrode for electrophysiological experiment was introduced into the Vc-I–II ipsilateral to the stimulation (P. 1.5 mm, L. 1.5–2.0 mm and H. 1.0–1.2 mm). The location of the cannula and electrode tip were verified histologically at the end of the experiment. Perfusion was carried out at a rate of 50 µl/min with artificial cerebrospinal fluid (128.5 mM NaCl, 3.0 mM KCl, 1.15 mM CaCl2, 0.8 mM MgCl2, 21.0 mM NaHCO3, 0.25 mM Na2HPO4, 3.4 mM glucose, 0.075 mM pargyline, 0.038 mM desipramine, 0.038 mM imipramine, 0.03 mg/ml bestatin, 50 mM captopril and 0.02 mM phosphoramidon). Perfusate samples were collected at intervals of 20 min in test tubes placed in ice. The samples were lyophilized and then their SP contents were radioimmunoassayed as described previously (7).

To determine the effect of various treatments on SP release, the mean values for the 3 pretreatment collections (each at 20-min intervals) were determined and used as the control values (100%).

Substances used

The following compounds were used: CP-96,345 (donated by Pfizer Pharmaceuticals, Inc., Groton, CT, USA); morphine hydrochloride (Sankyo Pharmaceutical Co., Tokyo); naloxone hydrochloride (Sigma Chemical Co., St. Louis, MO, USA); methysergide (donated by Sandoz Pharma AG, Basle, Switzerland); prazosin hydrochloride (Wako Pure Chemical Industries, Inc., Osaka); yohimbine hydrochloride (Wako Pure Chemical Industries, Inc.). [1211][Tyr8]SP was purchased from DuPont/New England Nuclear Research Products (Daichikagakuyakuhin, Inc., Tokyo).

Statistical analyses

The results shown in this manuscript are expressed as mean values±S.E. The statistical significance of difference between the groups was determined by Dunnett’s multiple comparison test. Student’s t-test was also used in the comparison between the two groups.

RESULTS

Effect of CP-96,345 on the potentials evoked by ST in the Vc-I–II

As shown in Fig. 1, the field potentials evoked by ST were found to be composed of two main components with latency of 4.34±0.17 msec (n=41, S.E.M.) and 9.41±0.17 msec (n=41, S.E.M.). The conduction velocities of the former component and the latter component are ca. 30 m/sec (fast component) and ca. 12 m/sec (slow component), respectively. In all cases of this experiment, the fast component was not changed by CP-96,345 (1–5 mg/kg, i.v.), whereas the slow component was significantly inhibited from 10 to 20 min after injection. The maximum inhibition for this compound was observed 10 min after i.v. injection in either investigated dose. At this time point, the amplitudes of field potentials were reduced to 74.0±2.9%, 49.0±5.9% and 37.0±4.6% after 1, 3 and 5 mg/kg administration, respectively, and gradually increased to the control level after 60 min. CP-96,344, an
inactive isomer, however, had no effect up to 5 mg/kg.

Effects of morphine alone or in combination with naloxone or methysergide on the potentials evoked by ST in the Vc-I-II

Pretreatment with morphine at a dose of 10 mg/kg (i.v.), which had produced a marked antinociceptive effect (15), significantly inhibited the slow component of field potentials, which occurred from 20 to 40 min after i.v. administration. At maximum inhibition, the amplitude of field potentials reduced to 15.7 ± 2.3% (n = 5, S.E.M.) of the control values. Naloxone (1 mg/kg, i.v.) or methysergide (5 mg/kg, i.v.) given 60 min after the administration of morphine very significantly antagonized this inhibitory effect of morphine (Fig. 2).

Effect of EAP alone or in combination with naloxone on the potentials evoked by ST in the Vc-I-II

The dispersion of relative amplitude of both components obtained in control animals at each measured time never exceeded 20%; therefore, when either or both components were reduced to less than 80% of the control amplitude after EAP stimulation, acupuncture was considered to have an effect. According to this criterion, animals were classified into the acupuncture-effective group and non-effective group. Acupuncture was effective in 8 of 11 animals observed. As shown in Fig. 3, in the acupuncture-effective group, EAP depressed the amplitude of the evoked potential of the slow component, but was without effect on the fast component. The significant depression induced by EAP was observed from 40 min to 80 min after the start of EAP. The slow component was depressed to a maximum of ca. 40% of the control amplitude. Intravenous injection of naloxone (1 mg/kg) reduced the inhibitory effect evoked by EAP in the slow component. As shown in Fig. 4, the relative amplitude of the slow component recovered from 60% to 80.1 ± 6.1% and 91.2 ± 4.9% of the control amplitude at 30 min and 50 min after injection, respectively.

Involvement of the monoaminergic system in the inhibitory effect evoked by EAP in the slow component

Intravenous injection of prazosin (5 mg/kg) or yohimbine (1 mg/kg) did not antagonize the depression induced by EAP, whereas methysergide (5 mg/kg) significantly inhibited the effect of EAP (Fig. 5). In all these experiments, the amplitude of the evoked potential of both components was unaffected by i.v. injection of drugs.
alone.

Effect of EAP on ST induced SP release in the Vc-I-ll

The SP concentration in the perfusate was initially high, but gradually decreased with time to a steady level 60 min after the start of the perfusion, which was maintained for up to 300 min. The SP level in the resting state, that is, in three 20-min fractions obtained in a 60-min period from 100 min after starting the perfusion to 160 min, was 0.014±0.002 pmol/20 min (n=45, S.E.M.). ST with a 40 V square wave evoked an increase in release of SP. The maximum increase was observed in the sample during ST (257.7±24.3%, n=23, S.E.M.), and this maximum increase of stimulus-evoked SP release was reduced to 145.4±23.3% (n=22, S.E.M.) by EAP. The inhibitory effect of EAP was significantly antagonized by naloxone (1 mg/kg, i.v.) injected 10 min before the beginning of EAP (327.4±81.3%, n=6, S.E.M.). In these perfusion experiments, the spontaneous release of SP from the Vc-I-II was unaffected by i.v. injection of drugs alone.

DISCUSSION

The trigeminal nucleus is considered to be a relay station for impulse transmission from the tooth pulp afferents to cells forming ascending pathways at higher levels of the brain (16, 17). It has been demonstrated that the tooth pulp afferents consist of Aβ and C fibers and that stimulation applied to these fibers elicits a relatively pure pain sensation (18). In the present electrophysiological experiments, ST produced potentials consisting of two main components: those with short latency derived from Aβ fibers and those with long latency derived from Aδ fibers. The potential corresponding to the conduction velocity of C fibers, however, was not observed. In this
A recent anatomical study indicates that a great portion of primary neurons innervating the incisor tooth pulp are myelinated fibers, because the size of their cell bodies range from 200 to 500 pm^2 and neurons less than 200 pm^2 were very rare (19, 20).

Fig. 3. Time course of effect of electro-acupuncture on the amplitude of the fast component (I) and the slow component (II) on the responses evoked by tooth pulp stimulation in the trigeminal nucleus caudalis. Electro-acupuncture was performed at 2 Hz and 5.4 V for 40 min at the time indicated by the solid line. < represents the time-course of change in the amplitude of the evoked potential obtained in the acupuncture-effective group. Values represent means±S.E.M. for 8 experiments. *P<0.05, compared with the relative amplitude in animals without electro-acupuncture stimulation (○) at the same time (Student's t-test for unpaired values).

Fig. 4. Time course of effect of naloxone on the inhibitory effect evoked by electro-acupuncture on the slow component. Naloxone (1 mg/kg, i.v.) was given 30 min after starting the application of electro-acupuncture. Values represent means±S.E.M. for 8 (○: control), 8 (△: acupuncture) and 8 (▲: acupuncture; naloxone, 1 mg/kg, i.v.) experiments. *P<0.05 and *P<0.05, compared with the relative amplitude in animals without electro-acupuncture stimulation (○) by Dunnett's test and with electro-acupuncture stimulation (●) by Student's t-test, respectively.

Morphine significantly inhibited the slow component of field potentials. This inhibitory effect was antagonized by naloxone or methysergide, suggesting that the slow component, reflecting the excitation of Aδ fibers, is involved in dental pain transmission and that the inhibitory effect of morphine is probably brought about through the activation of 5-HT pathways. With regard to the neurochemistry of synaptic transmission in the Vc-I-II, the component sensitive to morphine was also inhibited by CP-96,345, a potent and selective antagonist of SP (21, 22), and the release of SP was enhanced by ST. It is noteworthy that primary neurons innervating the mammalian tooth pulps, which are the finely myelinated and unmyelinated axons, project to the Vc-I-II (23–25); and SP-like immunoreactivity has been found in small-diameter primary afferent fibers of the tooth pulp and in the caudalpart of STN (5, 26–28). Furthermore, iontophoretically applied SP caused excitation of only neurons located in the trigeminal nucleus caudalis that responded to noxious cutaneous stimuli and/or to ST (6). These findings, and our present results, strongly support the idea that SP may play a role as a primary afferent transmitter...
or modulator in the transmission of dental pain messages.

In the present study, EAP stimulation to the Tsu-san-li showed an effectiveness of ca. 70% in the electrophysiological experiments; the stimulus-evoked long-latency response was markedly inhibited in ca. 70% of the animals given acupuncture. This result indicates that the individual variation exists in the effectiveness of acupuncture. In this respect, Richardson and Vincent (11) concluded that 50%–70% of patients with pain achieved clinically significant short-term pain relief, compared with the more commonly reported placebo response rate of ca. 30%.

Concerning the mechanisms of action of acupuncture, the stimulus-evoked long-latency responses and the increase in SP release were markedly inhibited by EAP, and systemic naloxone exerted antagonistic actions against EAP, suggesting that the opioid system is involved in a working mechanism of acupuncture. A number of studies on pain in recent years have indicated that two modulatory systems are controlling conduction of the ascending sensory message at the level related to the first synaptic relay stations in the trigeminal brainstem nuclei and spinal cord (29–32). One is an intrinsic mechanism associated with the segmental opioid system and the other is a descending monoaminergic system originating in the

Fig. 5. Time course of effect of methysergide (I), prazosin (II) and yohimbine (III) on the inhibitory effect evoked by electro-acupuncture on the slow component. Each compound was given 30 min after starting the application of electro-acupuncture. Panel I: Values represent means±S.E.M. for 8 (O: control), 8 (■: acupuncture; methysergide, 5 mg/kg, i.v.) experiments. *P<0.05 and *P<0.05, compared with the relative amplitude in animals without electro-acupuncture stimulation (O) by Dunnett’s test and with electro-acupuncture stimulation (■) by Student’s t-test, respectively. Panel II: Values represent means±S.E.M. for 8 (O: control), 8 (■: acupuncture) and 8 (▲: acupuncture; prazosin, 5 mg/kg, i.v.) experiments. *P<0.05, compared with the relative amplitude in animals without electro-acupuncture stimulation (O) by Dunnett’s test. Panel III: Values represent means±S.E.M. for 8 (O: control), 8 (■: acupuncture) and 8 (▲: acupuncture; yohimbine, 1 mg/kg, i.v.) experiments. *P<0.05, compared with the relative amplitude in animals without electro-acupuncture stimulation (O) by Dunnett’s test.
brainstem. In this respect, we have already reported that the transmission of dental pain in the Vc-I–II could be regulated through activation of the intrinsic enkephalinergic system (7) and a serotonin descending pathway originating in the nucleus raphe magnus (NRM) (8, 9).

In the electrophysiological experiments, the inhibitory effect of EAP was antagonized by methysergide, a 5-HT antagonist, but neither by prazosin, an α1-antagonist, nor yohimbine, an α2-antagonist. Moreover, the stimulus-evoked SP release was significantly inhibited by EAP, which was antagonized by pretreatment with methysergide as well as naloxone. These results suggest that 5-HT but not the noradrenergic system is mainly involved in the inhibitory effect evoked by EAP. It was previously demonstrated that local application of 5-HT inhibited the increase in the release of SP evoked by ST, and this inhibitory effect was antagonized by methysergide applied concomitantly to the superficial layers of the trigeminal nucleus (9). Anatomical studies, employing autoradiographic and immunocytochemical techniques, have demonstrated that 5-HT in the spinal dorsal horn and trigeminal nuclei is associated with nerve terminal systems originating from cell bodies located in the raphe nucleus (33, 34). We also observed that electrical stimulation of the raphe nucleus enhanced the release of 5-HT and inhibited release of SP (9). These findings, taken together with the present results, indicate that EAP may cause inhibition of stimulus-evoked SP release from the primary sensory afferent fibers by activating the descending serotonergic pathways.

With regard to involvement of the opioid system in the action of acupuncture, the present study showed that the inhibitory effect of EAP was antagonized by the pretreatment with naloxone and methysergide. We have also reported that systemic administration of morphine, similar to EAP (14), significantly inhibited the release of SP from Vc-I–II evoked by ST, and this inhibition was antagonized by local application of methysergide (8). Our present and previous results indicate that EAP initially activated opioid neurons which innervated the nucleus of the origins of the descending pathways such as NRM. In this connection, Yaksh and Tyce (35) reported that microinjection of morphine into the periaqueductal gray, which is relatively rich in opiate binding sites, evoked a release of 5-HT into the spinal perfusates parallel with the time lapse and levels of analgesia. Vasko and Vogt (36) found that similar microinjections into the vicinity of NRM caused a naloxone-reversible increase in 5-HT turnover in the dorsal horn of the spinal cord. In addition, numerous neuropharmacological studies suggest that opioid directly and/or indirectly enhances the activity of the bulbospinal serotonergic system and that behavioral analgesia is partly related to the inhibitory effects induced by the activation of such a descending system on the nociceptive message transmission at the spinal cord (37–39). These facts support the idea mentioned above.

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