Serotonin Receptor Subtypes Involved in Modulation of Electrical Acupuncture

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ABSTRACT—We examined the effects of intravenous injection of several serotonin (5-HT) antagonists on the inhibitory action of electro-acupuncture (EAP) against the nociceptive responses in the trigeminal nucleus caudalis in rabbits. The inhibitory effect of EAP was suppressed by pindolol, methysergide and ICS 205-930, whereas NAN-190 and ketanserin amplified the EAP effect. These results suggest that 5-HT₁, except 5-HT₁A, 5-HT₂, except 5-HT₂A, and 5-HT₃ receptors are positively involved in EAP-induced analgesia, whereas the activation of 5-HT₁A and 5-HT₂A receptors suppressively act on EAP-induced analgesia.

Keywords: Electro-acupuncture, Evoked potential, Serotonin receptor subtype

Although acupuncture has been a very common healing technique in the treatment of pain in the oriental world since ancient times, its detailed mechanism still remains unresolved. The spinal trigeminal nucleus (STN) is considered to be closely associated with the perception and transmission of orofacial sensory information (I). The STN is divided into three subnuclei, the oralis, interpolaris, and caudalis (2). Among these subnuclei, the subnucleus caudalis is thought to be most functionally and anatomically analogous to the medullary dorsal horn (3) and a site for the relay nucleus of the craniofacial nociceptive information to higher levels of the brain. We have already demonstrated that one of the mechanisms of analgesia induced by electro-acupuncture (EAP) is due to inhibition of the tooth pulp stimulus (ST)-evoked substance P release in the superficial layers of the trigeminal nucleus caudalis (SpVc-I,II) through activation of the descending serotoninergic systems linking up with opioidergic systems (4). With regard to serotonin (5-HT) receptors related to the spinal antinociception, it was demonstrated that different binding sites such as 5-HT₁, 5-HT₂ or 5-HT₃ were implicated in the analgesic effect of 5-HT (5–8). Therefore, in this study, we tried to elucidate which receptor subtypes of 5-HT are involved in the mechanisms of the effect of EAP.

The experiments were carried out on male rabbits weighing 2.5–3.0 kg. Under urethane anesthesia (1.0 g/kg, i.p.), the animal was inserted with an enamel-insulated bipolar electrode into the tooth pulp of the mandibular incisor, and then it was fixed on a stereotaxic apparatus. After the incision of the atlanto-occipital membrane of the cisterna magna, the tip of a monopolar recording stainless steel electrode was introduced into the SpVc-I,II ipsilateral to the tooth pulp stimulation (P. 1.5 mm, L. 1.5–2.0 mm and H. 1.0–1.2 mm). For noxious stimulation, the unilateral lower incisor pulp was stimulated electrically for 100 sec with a square wave (intensity of 7–15 mA, frequency of 2 Hz, duration of 0.1 msec). The summed evoked potentials were obtained by adding 200 responses by means of computer average transient (MEB-4108; Nihon Kohden, Tokyo). Two stainless-steel needles for acupuncture (gauge No. 3 and 48 mm in length, diameter of 0.2 mm) were inserted into tibial muscle (inter-electrode distance of 5 mm, depth of 5 mm), relevant to ‘Tsu-San-li’ in the human acupuncture points. EAP was applied for 40 min at a low frequency (2 Hz) and a high intensity, which elicited a slight twitch (5.4 V), using an apparatus for low frequency current therapy (Lasper CS-504; Kanaken Medical Instruments, Tokyo).

To determine the influence of EAP on the responses evoked by ST and the effect of antagonists of 5-HT-receptor subtypes on analgesia induced by EAP, the amplitude of field potentials evoked by ST before EAP stimulation was used as the control values (100%). 5-HT-antagonists were intravenously administered 10 min before the ending of EAP stimulation. ICS 205-930
(5-HT₃ antagonist) and methysergide (non-selective 5-HT₁ or 5-HT₂ antagonist) were generously donated by Sandoz Pharma, Ltd., Basle, Switzerland. NAN-190 (5-HT₁A antagonist), ketanserin (5-HT₂A antagonist) and pindolol (5-HT₁B antagonist) were obtained from Research Biochemicals Incorporated (Natick, MA, USA). The results obtained were expressed as the mean ± S.E., and the statistical significance of difference between groups was determined by Dunnett’s multiple comparison test. Student’s t-test was also used in the comparison of two groups. The criterion for statistical significance was P < 0.05 in all cases. The ethical guidelines for investigations of experimental pain in conscious animals were followed, and all experiments were approved by the Animal Care and Use Committee of our Institution.

As shown in Fig. 1, the field potentials evoked by ST were found to be composed of two main components with a latency of 4.3 ± 0.2 msec (n = 6) and 9.4 ± 0.2 msec (n = 6). 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. As mentioned previously, 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, EAP was considered to have an effect. According to this criterion, animals were classified into the EAP-effective group and non-effective group. EAP stimulation depressed the amplitude of the evoked potential of the slow component in ca. 70% of the total animals investigated, but was without effect on the fast component. The significant depression evoked by EAP was observed from 40 to 80 min after beginning EAP stimulation. The slow component was depressed to a maximum of ca. 39.7% of the control amplitude 60 min after the beginning of EAP stimulation. Electrical stimulation of the abdominal muscle as a non-acupuncture point did not produce any depression of the amplitude of the slow component.

![Fig. 1. Effect of electro-acupuncture (EAP) on the responses evoked by tooth pulp stimulation in the trigeminal nucleus caudalis. Typical evoked responses obtained before (1), immediately after (2) and 100 min after (3) EAP. The potentials evoked by tooth pulp stimulation were composed of two main components with conduction velocities of ca. 30 m/sec (fast component) and ca. 12 m/sec (slow component). The open and closed arrows indicate the fast and slow component, respectively.](image-url)
component. The animals of the EAP-effective group were used for 5-HT-antagonist experiments. Intravenous injection of pindolol (5 mg/kg), methysergide (5 mg/kg) or ICS 205-930 (1 mg/kg) significantly inhibited the depression induced by EAP. The relative amplitude of the slow component recovered from $45.5\pm 5.4\%$ to $85.6\pm 5.6\%$, $74.5\pm 3.0\%$ and $98.5\pm 3.8\%$ of the control amplitude at 10 min after injection of pindolol, methysergide and ICS 205-930, respectively (Fig. 2). On the other hand, intravenous injection of NANN-190 (3.5 mg/kg) or ketanserin (2.5 mg/kg) did not antagonize the depression induced by EAP, but significantly enhanced the effect of EAP (Fig. 3). In all these experiments, the amplitude of the evoked potential of both components was unaffected by i.v. injection of drugs alone.

A number of studies on pain in recent years have suggested 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 nuclei and spinal cord (9–11). One is the intrinsic mechanism associated with the segmental opioid system and the other is a descending monoaminergic system originating in the brainstem. In this connection, we have already reported that the transmission of dental pain in the SpVc-I,II could be regulated through activation of the intrinsic enkephalinergic system (12) and a 5-HT descending pathway originating in the nucleus raphe magnus (NRM) (7, 13). Concerning the mechanism of analgesia induced by EAP, we also have observed that EAP significantly inhibited the amplitude of the slow component accompanied by evoked potential at the SpVc-I,II, and this effect of EAP was antagonized by the pretreatment with methysergide (non-selective antagonist for 5-HT1 and 5-HT2) or naltrexone but not prazosin ($\alpha_1$-antagonist) or yohimbine ($\alpha_2$-antagonist) (4). These findings support the idea that the 5-HT system is involved in manifestation of analgesia induced by EAP at the first synaptic relay station in the trigeminal sensory nucleus. With regard to 5-HT-receptor subtypes, in the last decade, at least seven distinct 5-HT-receptor subtypes: 5-HT1, 5-HT2, 5-HT3, 5-HT4, 5-HT5, 5-HT6 and 5-HT7 receptors have been identified. Concerning 5-HT-receptor subtypes related to the spinal antinoceptive, Crisp et al. (6) observed that the 5-HT3 site is primarily responsible for the analgesic effect of 5-HT in rats. Chojnacka Wojciech et al. (7) concluded that the analgesia induced by m-chlorophenylpiperazine, a non-selective 5-HT-receptor agonist, is mediated by 5-HT3 receptors in mice. On the other hand, Alhaider et al. (8) reported that the spinal descending serotonergic system was closely related to the 5-HT3 receptor in mice. These findings, and our results obtained with rabbits demonstrating that the inhibitory effect of EAP to ST-evoked potential was antagonized by pindolol (5-HT1B antagonist), methysergide (non-selective 5-HT1 or 5-HT2 antagonist) and ICS 205-930 (5-HT3 antagonist), whereas NANN-190 (5-HT1A antagonist) and ketanserin (5-HT2A antagonist) enhanced the inhibition of amplitude induced by EAP strongly support the idea that the involvement of 5-HT in the hyperalgesic response may differ among 5-HT-receptor subtypes. Namely, 5-HT1 and 5-HT2, except 5-HT1A and 5-HT2A, and 5-HT3 receptors may be involved in inhibitory regulation of dental pain transmission induced by activating the descending serotonergic pathway. With regard to the

![Fig. 2. Time course of the effects of methysergide, ICS 205-930 and pindolol on inhibitory effect induced by electro-acupuncture (EAP) on the slow component. Each compound (●: methysergide, 5 mg/kg, i.v.; ▲: ICS 205-930, 1 mg/kg, i.v.; ◆: pindolol, 6 µg/g, i.v.) was administered 30 min after starting the application of EAP (indicated by arrow). Values represent means ± S.E. for 6 experiments. *P<0.05, compared with the relative amplitude in animals with EAP (■) by Dunnett's test.]  

![Fig. 3. Time course of the effects of NANN-190 and ketanserin on inhibitory effect induced by electro-acupuncture (EAP) on the slow component. Each compound (●: NANN-190, 3.5 mg/kg, i.v.; ▲: ketanserin, 2.5 mg/kg, i.v.) was administered 30 min after starting the application of EAP (indicated by arrow). Values represent means ± S.E. for 6 experiments. *P<0.05, compared with the relative amplitude in animals with EAP (■) by Dunnett's test.]
effect of NAN-190, this effect depends on presynaptic 5-HT$_{1A}$ receptors (autoreceptors) that exert an inhibitory control on nerve activity and 5-HT synthesis/release. In this connection, Hjorth (14) reported that 5-HT$_{1A}$-autoreceptor blockade potentiated the ability of the 5-HT-reuptake inhibitor citalopram to increase nerve terminal output of 5-HT in rats. This is in accordance with our results, although the species used for the experiments are different. On the other hand, ketanserin is known to be the most selective antagonist of the 5-HT$_{2A}$ receptor. It has been observed that the 5-HT$_{2A}$-mRNA transcript is expressed in the spinal cord tissue, although there is, as yet, little evidence for physiological and biological activity on the 5-HT$_{2A}$ receptor of the spinal cord and trigeminal nucleus (15). Our current results suggest that the 5-HT$_{2A}$ receptor may be involved in modification of the transmission of the pain sensation at the first relay station through activation of excitatory pathways, and 5-HT$_{1A}$ and this receptor activation may suppressively act on EAP-induced analgesia.

REFERENCES