

The Effects of Transcranial Electrical Stimulation on Anaesthesia and Analgesia in Rats

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ABSTRACT. In this study, we determined the effects of transcranial electrical stimulation (TCES) on the anaesthetic requirements of thiopental and the analgesic requirements of remifentanyl, in rats. The experiments were performed on 120 albino male Wistar rats, which were randomly allocated to four groups (n=30). (Thiopental, Thiopental+TCES, Thiopental+Remifentanyl, and Thiopental+Remifentanyl+TCES). Animals were anaesthetized with thiopental, and 15 min later, remifentanyl was injected to rats in the Remifentanyl groups. TCES was started in the stimulated groups 20 min after thiopental administration. Rats were stimulated 5 times for this experiment. The times for recovery, herein called Cognition Recovery Time and Motion Recovery Time were measured. Cognition Recovery and Motion Recovery Times were not affected by the stimulation. Analgesia was assessed using the wet tail-flick latency (TFL). In the Thiopental group, the analgesia level returned to control values on the 35th min. In the Thiopental+Remifentanyl group, the analgesia level returned to control values on the 50th min. In the Thiopental+ TCES group, the analgesia level reached the peak value on the 65th min. In the Thiopental+Remifentanyl+TCES group, the analgesia level reached the peak value on the 35th min and analgesia remained high during the 90 min after cessation of the stimulation. The analgesic potency for the Thiopental+Remifentanyl+TCES group was increased by 30–40% when compared with the prior TFL values, 160% when compared with the control group, and 50–75% when compared with Thiopental+ TCES group on the 35th min ($P<0.001$). In conclusion, TCES markedly decreases the anaesthetic and analgesic requirements for thiopental and remifentanyl in rats.

KEY WORDS: remifentanyl, thiopental, transcranial electrical stimulation.

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Since the beginning of the century, low frequency electrical currents have been used to provide analgesia and anaesthesia in both animals and humans. This system is called “Transcranial Electrical Stimulation (TCES)” [7]. However, low frequency electrical currents are always associated with severe side effects such as apnoea, cardiac accidents, and convulsions [17]; therefore, different stimulation methods have been developed. High frequency TCES consists of low frequency trains of high frequency current. This allows an increase in current intensity without reduction of tissue conductivity, which is normally associated with low frequency currents. This type of TCES is called “Limoge’s Currents”. Recent experiments have shown that TCES significantly potentiates the analgesic effects of opioids and anaesthetics in humans and rats. Undesirable side effects attributable to TCES have been reported in these studies [1, 2, 15–18]. However, the mechanisms and neurobiological substrates of TCES remain unknown.

Currently used anaesthetic agents may be associated with circulatory and respiratory depression (in pulmonary disease) and delay (in hepatic and renal disease) in recovery time. Additionally, intravenous anaesthetic agents like barbiturates do not have any analgesic effect [4]. The analgesic effects of opioids are generally sufficient, but side effects such as delay in recovery time and circulatory depression and apnoea may occur even with optimal doses [12]. In this study, we chose remifentanyl as a short acting opioid and thiopental, which has a non-analgesic property, to test the effects of opioids in respect to TCES. The preliminary

results obtained from TCES in the field of clinical anaesthesia seem to be promising. By decreasing anaesthetic and analgesic requirements, a better quality of anaesthesia, a faster recovery from anaesthesia, and a longer-lasting post-operative analgesic period can be obtained, especially in high-risk patients [1, 2, 14–16].

The aim of this study was to determine the influence of TCES on the analgesic effects of short-acting remifentanyl and on the anaesthetic effects of thiopental and in rats.

MATERIALS AND METHODS

Experiments were performed on 120 albino male Wistar rats (Hifzissiha Institute, Ankara, Turkey) weighing 140–180 g. Rats were housed on a 12:12 hr alternating light/dark cycle with food and water *ad libitum*. They were randomly allocated to four groups, each consisting of 30 animals.

Animals were anaesthetized with 2 mg kg⁻¹ thiopental (T) injection (sedation dose) intraperitoneally. Fifteen minutes later, remifentanyl (R) was injected intraperitoneally (low dose; 0.5 µg kg⁻¹) to rats in the remifentanyl groups [6]. After anaesthesia, each rat was confined in a transparent pliant retention box, which impaired deambulation but left the head and tail free. Venous inoxidizable needles were used to make up the electrodes. The frontal electrode was placed on the cranium between the eyes on the mesopic suture and the two posterior electrodes were placed behind the mastoid bones on each side. Electrodes were connected to the electrical generator with thin, flexible cables.

The stimulation was provided by a homemade generator. It was calibrated with a digital oscilloscope (Multimeter, Hewlett Packard 973 A, U.S.A.®). In our study, the electrical currents (Limoge's Current) consisted of low-frequency trains of high frequency current (high frequency intermittent bursts of bi-directional balanced currents, 166 kHz; low frequency, 4 msec at 100 Hz; current intensity, 100 mA). In each HF cycle, the current was positive for 2 μ sec and negative for 4 μ sec [11]. The frontal electrode was connected to the negative pole of the generator and received a negative impulse of weak intensity (33 mA, long duration 4 μ sec), and the two posterior electrodes were connected to the positive pole and received a positive impulse of high intensity (67 mA, short duration 2 μ sec). TCES was performed 5 times in the stimulated groups (T+TCES group, T+R+TCES group) after the first tail-flick latency test on the 20th min. Each stimulation period lasted 15 min with approximately 20–23 sec resting periods. Total stimulation time was 75 min. The electrodes were also placed in the T group and the T+R group, but the rats were not stimulated.

Analgesia was assessed using the wet tail-flick latency (TFL) [24]. The distal third of the rat's tail was immersed in a thermostat water bath (50°C) and the time before the tail reflex of withdrawal (second) was measured. For TFL measurements, the rats were not stimulated for a short period and the first tail movement time was recorded. Whenever the tail withdrawal reflex did not arise within 23 sec, the tail was manually withdrawn from the water to avoid tissue damage. To measure the physiologic TFL (without application of TCES and drugs), the TFL test was done on 5 rats from each group (a total of 20) a week before the experiment (control group). The non-stimulated (after drugs) and stimulated groups (after drugs and before TCES) received the first TFL test on 20 min after the administration of thiopental. The following measurements were done between TCES application at the end of each rest period. After TCES was stopped, the measurements were done within 30 min intervals. Measurements were continued in each group until the normal TFL value was reached.

The time for recovery (minutes) of the muzzle and eye movements, herein called Cognition Recovery Time (CRT), and the time for recovery of free limb movements or deambulation (min), herein named Motion Recovery Time (MRT) were measured in the test groups. The time for CRT was measured up to the first movement on the muzzle or of the eyes after drug administration. For MRT, the procedure was the same but the time for the first paw movement was recorded.

The values obtained by measurements were compared in terms of mean and standard deviation. Kruskal Wallis one-way analysis of variance was used for comparison between the groups; Friedman two-way analysis of variance was used for comparison within the groups for time dependent differences.

Our study was approved by the Committee for Research and Ethical Issues of Ankara University Veterinary Faculty.

RESULTS

This experiment was performed with 4 independent groups of rats (n=30). All results are expressed as mean \pm SD.

No major adverse effects were observed throughout the procedures. The times for CRT were found to be 20.67 ± 3.43 min in the T group, 22.47 ± 2.56 min in the T+TCES group, 26.52 ± 2.21 in the T+R group, and 25.88 ± 3.64 min in the T+R+TCES group. The times for MRT were 27.56 ± 2.26 min in the T group, 26.78 ± 3.78 min in the T+TCES group, 32.65 ± 2.88 min in the T+R group, and 31.83 ± 3.82 min in the T+R+TCES groups. The times for motion, recovery and cognition recovery were found to be longer in groups with remifentanyl ($P < 0.001$).

In the control group the physiologic TFL was found as 5.04 ± 0.87 sec one week before the experiments. The test groups differed significantly from the control group ($P < 0.001$) (Fig. 1). TFL test results at the 20th min were similar in the T and T+TCES groups, and in the T+R and T+R+TCES groups. In the T group, on the 20th min following thiopental injection, the withdrawal reflex time (10.90 ± 1.15 sec) was almost two times longer than the control group ($P < 0.001$) and returned to the control values on the 35th min. In the T+R group, on the 20th min, the withdrawal reflex time (14.24 ± 1.01 sec) was significantly longer than the control and T groups ($P < 0.001$) and returned to the control value on the 50th min. In the T+TCES group the analgesia level (10.63 ± 0.99) on the 35th min was similar to the measurement of the unstimulated TFL values on the 20th min. It reached a peak value (14.61 ± 0.87 sec) on the 65th min and persisted throughout the stimulation period. In the T+R+TCES group the analgesic effect had increased (19.76 ± 0.88 sec) by the 35th min with regard to the unstimulated TFL values on the 20th min and persisted throughout the stimulation period. In the T+R+TCES group, analgesic response after stimulation was seen earlier and was more potent than in the T+TCES group ($P < 0.001$).

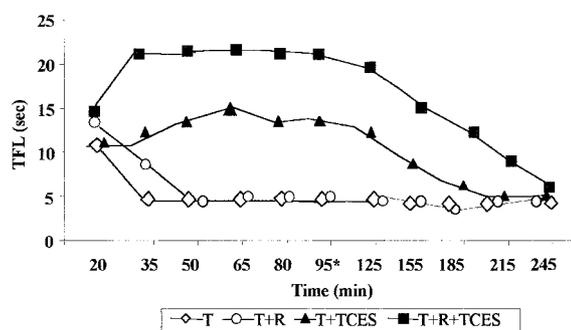


Fig. 1. Time-dependent change of TFL in stimulated and non-stimulated groups. * TCES was stopped at the 95th min. ▲ In the T+TCES group, at the 50th -155th min, TFL times were significantly longer than in the T and T+R groups. ■ In the T+R+TCES group, at the 20th -185th min, TFL times were significantly longer than in the other test groups ($P < 0.001$).

The analgesic potency for the T+R+TCES group increased 30–40% when compared with the prior TFL values, 160% when compared with the control group, and 50–75% when compared with T+TCES group on the 35th min. The difference between other groups on the 15th, 30th, 45th, 60th and 75th min after stimulation was significant ($P<0.001$). Analgesia was also high during the 90 min after stimulation was discontinued in the T+R+TCES group, and it was significantly different from the other groups ($P<0.001$). In stimulation groups, after discontinuing the stimulation we observed a progressive decrease in the measurements done at 30 min intervals. Control withdrawal reflex time was reached after 90 min in the T+TCES group, and after 120 min in the T+R+TCES group after cessation of the stimulation.

DISCUSSION

Thiopental is a barbiturate, which needs a time shorter than 30 min for brain action when used intravenously in humans. Thiopental when used intraperitoneally in rats needs 30–40 min for brain action [4, 13]. Remifentanyl is a new, ultra-short acting μ -opioid agonist that is metabolised by plasma and tissue esterases [3]. The elimination half-life of remifentanyl is approximately 10–20 min [21]. Intraperitoneally administered remifentanyl reaches its peak effect in 5 min and the duration of action is approximately 10 ± 5 min [13]. As reported by Wilhelm and colleagues [22] remifentanyl decreases the requirements for induction of anaesthesia with thiopental. Mathematical analysis of Limoge's Currents based on Fourier series indicates that a complex pattern of stimulation allows deep penetration of the electric field into the brain without macroscopic damage in the cerebral parenchyma [1].

TCES didn't effect the recovery time of thiopental and remifentanyl in our study. From the anaesthetic point of view, our results indicate that TCES significantly decreases opioid and anaesthetic requirements in animals and humans without effecting spontaneous behaviours [15–18]. In our study, there were no significant differences among groups of T and T+TCES, and among groups of T+R and T+R+TCES for CRT and MRT. The stimulation that we applied had no effect on recovery times. Thus, we achieved a longer administration period with no side effects.

Recent experiments have shown that 30–40% of TCES potentiates the analgesic effect of N_2O [13], and 50–80% of TCES decreases opioid requirements during neuroleptanaesthesia in humans [15]. Stinus and colleagues [18], performed a study in rats, and they observed that morphine provided a 61% increase in TFL when compared with control values, and this increased reaching 171% with the addition of TCES. In our study, analgesia was increased 160% in the T+R+TCES group when compared with control group. Some other studies have shown that anaesthetics with low analgesic properties can also potentiate analgesia. For example, TCES markedly (44%) decreased the anaesthetic requirements for halothane in rats [11]. In our study,

application of TCES, in the thiopental and remifentanyl groups (more in the opioid receiving group) potentiated analgesia.

There are different perspectives about the starting time of stimulation to potentiate the anaesthetic and analgesic effects of TCES. Stinus and colleagues [18] have reported that the analgesic effect of subcutaneous morphine was potentiated by starting TCES 3 hr before injection. Different observers have achieved successful analgesia using TCES 30 min before injection [10, 14, 23]. In addition, the analgesia started on the 20th min of low frequency stimulation ($10 \mu A$) in drug-free rats. It reached the maximal effect on the 30th min and lasted 180–200 min after cessation of the stimulation [14, 23]. In our study, we used TCES with high frequency and we started stimulation on the 20th min after drug administration. We administered remifentanyl 15 min after thiopental in order to minimise the potentiating effect of remifentanyl. The difference between the T and T+R groups in reaching the normal retreat reflex time was 15 min. The level of analgesia provided with thiopental in the T+TCES group remained the same throughout the stimulation. The level of analgesia in the T+R+TCES group was potentiated by stimulation and remained high until the end of the stimulation. Analgesia lasted for 90 min in the T+TCES group and 120 min in the T+R+TCES group after cessation of the stimulation. This may imply that both of the agents are good enough to potentiate analgesia and anaesthesia with a 10 min stimulation, which was started before the end of the brain activity. In Skolnick's and Wilson's studies, it was shown that a 20 min TCES with low frequency application without anaesthetic and analgesic drugs could cause appropriate analgesia in rats [14, 23]. In our study, we reduced the time for TCES to 10 min with the help of the analgesic, anaesthetic and high frequency current used. In the field of clinical anaesthesia, it is important to reach proper analgesia and anaesthesia with stimulation after induction, because longer lasting stimulations before induction may cause anxiety for awake patients. Stimulation after sedation (with lower doses of thiopental or remifentanyl) is tolerable for patients. Thus, a sufficient level of anaesthesia and analgesia during surgery might be obtained with the use of lower doses of anaesthetic and analgesic agents if the stimulation given after sedation increases the effect of agents within 10 min as in the case of our study and prolongs its effect.

There are many hypotheses regarding the mechanism of TCES. Stimulation may alter the kinetics of the drug and change the blood-brain barrier permeability, increasing the penetration of the drug into the brain. It may create a new steady state in brain activity, which might be revealed by a pharmacological challenge [9–11, 18]. The stimulation of the periaqueductal gray matter induces highly specific naloxone-reversible pain suppression that is associated with increased immunoreactive β endorphins in ventricular fluid [8]. Enkephalinase inhibitors increase the analgesia caused by TCES. The opioid antagonists such as naloxone reverse this effect. It is believed that endogenous opioids take part

in this mechanism [7, 10, 11]. Moreover, noradrenergic [20], serotonergic or both types of fibers [19] have been shown to play a key role in the medication of analgesia at the spinal level. The hypothalamus and thalamus can be considered as one of the pain modulation sites [5]. Our study supports the hypothesis that stimulation causes an electrical hyperpolarizing action on the hypothalamus, as TCES potentiates analgesia not only in opioid group drugs but also in hypnotic group drugs as well.

In conclusion, TCES markedly decreased the anaesthetic and analgesic requirements for thiopental and remifentanyl without effecting recovery times in rats. This effect appears to be related to the creation of a new steady state in brain activity and to the release of enkephalins from brain structures, enhancing opioid analgesia. In clinical application, the use of lower doses of anaesthetic agents would minimize side-effects in both the operative and post-operative periods. Further clinical and fundamental research is needed to evaluate TCES and account for its action.

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