

Transcranial Electrical Stimulation with Limoge's Currents Decreases Halothane Requirements in Rats

Evidence for the Involvement of Endogenous Opioids

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Transcutaneous cranial electrical stimulation with Limoge's currents has been shown to facilitate anesthesia/analgesia in surgical patients. However, the neurobiologic substrate of this effect remains unknown. The present study was designed to analyze the influence of transcranial electrical stimulation (TCES) on halothane requirements in rats and the contribution of the central endogenous opioid, α_2 -adrenergic and 5-hydroxytryptamine (5-HT₁ and 5-HT₂) serotonergic systems to this effect. The influence of TCES on the MAC of halothane (MACH) and its reversibility by a subcutaneous 2 mg/kg naloxone injection were first determined in 20 rats using a randomized blinded protocol. MACH was decreased markedly in stimulated animals (TCES, n = 10) in comparison with sham-operated nonstimulated rats (controls, n = 10): MACH = 0.60 ± 0.15 , mean \pm SD, versus 1.07 ± 0.05 vol%, $P < 0.001$. In TCES animals, naloxone administration restored MACH values to the levels of controls but failed to affect MACH in controls. The influence of the duration of TCES applied prior to MACH determination was further investigated in 30 animals. The magnitude of MACH reduction was significantly increased with the cumulative duration of stimulation. For each duration of stimulation tested, administration of a 5- μ g intracerebroventricular (icv) dose of the enkephalinase inhibitor thiorphan significantly enhanced TCES effects ($P < 0.05$). Finally, the icv administration of a 15- μ g naloxone dose appeared to reverse completely the MACH reduction elicited by TCES (n = 8, $P < 0.01$). In contrast, neither the α_2 -adrenergic (norepinephrine) blocker yohimbine (30 μ g, n = 8) nor the nonselective serotonergic (5-HT) antagonist methysergide (30 μ g, n = 8) or the selective 5-HT₂ antagonist ritanserin (30 μ g, n = 8) given icv were effective in this regard. Taken together, these results indicate that TCES significantly reduces halothane requirements in rats and support the involvement of the endogenous opioid, but not monoaminergic systems, in this effect. (Key words: Anesthetic technique, transcranial electrical stimulation: Limoge's currents. Anesthetics, volatile: halothane. Antagonists, opioid receptors: naloxone. Antagonists, serotonergic receptors: methysergide; ritanserin. Inhibitors, enkephalinase: thiorphan. Potency, anesthetic: MAC. Sympathetic nervous system, α_2 adrenergic antagonists: yohimbine.)

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ATTEMPTS HAVE BEEN MADE during the past 20 yr to develop noninvasive methods of transcutaneous electrical stimulation for facilitating anesthesia in humans.^{1,2} However, severe cardiac, respiratory, and neurologic adverse effects were reported in these studies, which challenged the safety of the different patterns of stimulation used. Transcranial electrical stimulation (TCES) is a special form of electrical stimulation consisting of high-frequency alternating balanced currents, called Limoge's currents, delivered through stimulating electrodes located at specific areas of the head. Recent experiments have shown that TCES significantly potentiates the analgesic effect of opioids in rats.³ The preliminary results obtained with TCES in the field of clinical anesthesia seem also to be promising, since this particular pattern of stimulation increases by 30–40% the potency of nitrous oxide and significantly reduces opioid requirements during minor surgical procedures.^{4,5} Basically, no undesirable side effects attributable to TCES have been reported in these clinical studies.

However, the neurobiologic substrate of TCES effects remains unknown. It has been suggested that TCES might elicit the release of endogenous opioids such as enkephalins and endorphins, which are located in various brain structures.⁶ Indeed, these peptides very likely participate in analgesic processes. For instance, it has been shown that a peripheral noxious stimulus, such as electrical stimulation of the tooth pulp, elicits a long-lasting increase in the levels of met-enkephalins in the cerebrospinal fluid of anesthetized cats.⁷ On the other hand, stimulation of the periaqueductal gray matter induces highly specific naloxone-reversible pain suppression that is associated with increased immunoreactivity for β endorphins in ventricular fluid.^{8,9} Interestingly, electrical stimulation of the periaqueductal gray matter (which is rich in neurons containing endogenous opioids) has been shown to decrease halothane requirements in humans.¹⁰

However, numerous data indicate that other central neuronal systems, such as the monoamine-containing fibers, also participate in anesthetic/analgesic processes. In fact, noradrenergic (norepinephrine [NE]),^{11–13} serotonergic (5-hydroxytryptamine [5-HT]),¹⁴ or both types of fibers^{15–18} have been shown to play a key role in the mediation of analgesia at the spinal level. Selective destruction of the cell bodies containing 5-HT or NE results in

brain and cervical medulla following an icv injection of 10 μ l Pontamine Skye Blue solution. This infusion was performed through the cannula immediately before the animals were killed by a pentobarbital overdose. The data obtained with the tips of the cannulae located outside the cisterna magna were excluded from the study.

DETERMINATION OF HALOTHANE REQUIREMENTS

Halothane requirements were assessed by determination of the minimum alveolar concentration of halothane (MACH) using a classical up-and-down tail-clamp technique.²⁴ Briefly, anesthesia was induced in an induction chamber using a 3% inspired fraction of halothane (F_{I_H}) (Fluothane, ICI Pharma, Cergy, France). Once tracheostomy had been performed, the F_{I_H} was reduced to 1.3%, and ventilation was controlled with a mechanical rodent ventilator (PHYMEP Sarl, Paris, France) at about 60 breaths per min, 0.9–1 ml/100 g body weight, 100% oxygen. All rats were in the supine position and equilibrated to the 1.3% F_{I_H} during 45 min. Rectal temperature was controlled by servomechanism to 37° C with a homeothermic warming blanket (Harvard Apparatus, South Natick, MA). The left carotid artery was cannulated for arterial blood gas measurements, and ventilation was adjusted when appropriate to maintain P_{aCO_2} between 35 and 40 mmHg.

Halothane was administered using a continuous-flow vaporizer (Fluotec 3, Ohmeda, England), and the end-tidal concentration of halothane was monitored with a calibrated infrared analyzer (Cosma 815, France). Immediately after a measurement of end-tidal concentration of halothane, a 6-inch hemostat was applied to the base of the rat tail to full ratchet lock. The test was considered positive when a motor response of the head or hind limbs to this maximal noxious stimulus was observed within 60 s. The F_{I_H} was then changed up or down by 0.2% absolute and the test repeated every 15 min until the highest end-tidal halothane concentration allowing movement and the lowest preventing movement were determined. MACH was considered the midway point between these two values.

The reversibility of TCES effects was tested as follows: once MACH had been determined, the F_{I_H} was maintained at 1.1% for 15 min. Thereafter, injection of the antagonist was performed, and MACH was again measured as described above. For potentiation of TCES effects by the enkephalinase inhibitor thiorphan, the same protocol was used, but the F_{I_H} was maintained at the highest value allowing movement in response to the 60-s tail-clamp before the icv administration of thiorphan.

EXPERIMENTAL DESIGN

In the first set of experiments, the effects of TCES on MACH and their reversibility by systemic administration

of naloxone were investigated. Twenty rats were connected to the electrical generator and randomly allocated to be either stimulated ($n = 10$, TCES group) or not ($n = 10$, control group). A pair of animals (one in each group) was tested on each session by a blinded investigator. In the TCES rats, stimulation was initiated 10 h before the onset of the study period and still applied throughout the experiment. In all animals, MACH was determined prior to and 15 and 30 min following a subcutaneous injection of 2 mg/kg naloxone.

In the second set of experiments, the influence of the duration of stimulation upon TCES effects and the potentiation of these effects by thiorphan were addressed. Therefore, TCES was started at different times prior to tracheostomy: either 10 h ($n = 6$) or 4 h ($n = 6$) or 1 h ($n = 6$). Six additional animals were challenged with a 30-min period of stimulation, which was initiated once MACH was measured. In all of these rats, MACH was again determined following an icv 5 μ g thiorphan dose. Since thiorphan exhibits by itself antinociceptive properties,²³ this dose was chosen according to a preliminary dose-response curve that indicated that this dose was the highest tested that failed to decrease MACH in nonstimulated animals ($n = 6$).

In the third set of experiments, the antagonism of TCES effects by icv administration of naloxone (15 μ g, $n = 8$), yohimbine (30 μ g, $n = 8$), ritanserin (30 μ g, $n = 8$), and methysergide (30 μ g, $n = 8$) was studied. Therefore, MACH was determined before and following infusion of each of these compounds in animals stimulated 10 h prior to tracheostomy. The doses used for naloxone, yohimbine, methysergide, and ritanserin icv injection were selected on the basis of their effectiveness in specifically antagonizing antinociception following icv or intrathecal administration. Moreover, their pharmacokinetics and pharmacodynamics were suitable to the present protocol scheduled for MACH determination.^{16,25–28}

STATISTICAL ANALYSIS

Differences between mean values were assessed by the analysis of variance followed by the unpaired Student's test. The Wilcoxon's paired test was used for paired comparison of small samples. A P value < 0.05 was considered the threshold for significance.

Results

All results are expressed as mean \pm SD. No major adverse effect in terms of mortality was observed. Non-quantitative observations indicated that TCES did not affect the behavior of the rats. More precisely, no obvious modifications in eating, drinking, or sleeping were occurred in comparison with control rats when TCES animals were observed in their cages. The only manifestation

associated with either a 4- or a 10-h period of stimulation prior to tracheostomy was that the rats were particularly quiet in their cages and easy to manipulate. However, catalepsy was not present, and their tail-pinch reaction was vigorous. The righting reflex as well as the startle reflex were always preserved.

The results of the first set of experiments are shown in figure 2. The mean value for MACH in control animals was 1.07 ± 0.05 vol%. MACH was decreased in TCES animals when compared with controls ($\text{MACH} = 0.60 \pm 0.15$ vol%, $P < 0.001$). This effect was completely reversed by a 2-mg/kg subcutaneous naloxone dose, which restored MACH to control values ($\text{MACH} = 1.03 \pm 0.10$ vol%), whereas naloxone did not affect MACH in the nonstimulated animals ($\text{MACH} = 1.05 \pm 0.08$ vol%; fig. 2).

The results of the second set of experiments are displayed in figure 3. The cumulative durations of stimulation were 12.3 ± 0.5 , 6.4 ± 0.6 , and 2.8 ± 0.5 h in the animals for which stimulation was initiated 10, 4, and 1 h before tracheostomy, respectively. It was 1.5 ± 0.3 h in the rats stimulated for 30 min from the first determination of MACH. In the second and the third sets of experiments, the mean MACH values obtained in the rats stimulated for 10 h before tracheostomy were pooled together and compared to those obtained in the first set of experiments. No significant difference could be detected between these values ($\text{MACH} = 0.65 \pm 0.13$ vol% vs. 0.60 ± 0.15 vol%, $P > 0.05$). The mean MACH value was significantly different from controls in the animals for which the following cumulative durations of stimulation were applied: 2.8 ± 0.5 h ($\text{MACH} = 0.75 \pm 0.14$, $P < 0.01$) and 6.4 ± 0.6 h ($\text{MACH} = 0.68 \pm 0.12$, P

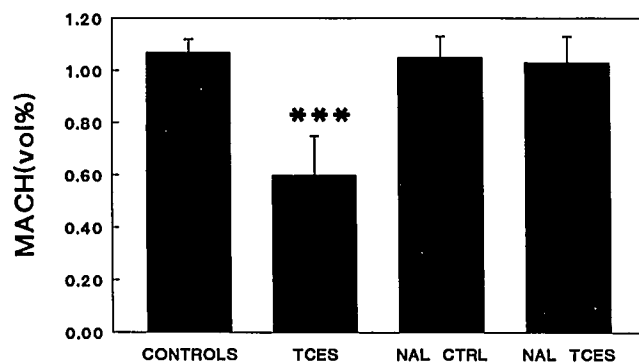


FIG. 2. Effect of TCES with Limoge's currents on halothane requirements in rats. Results are expressed as mean \pm SD. In the TCES group, stimulation was initiated 10 h prior to tracheostomy. TCES induced a significant decrease in the MAC of halothane (MACH) in comparison with controls (*** $P < 0.001$). Subcutaneous injection of a 2-mg/kg naloxone dose restored the mean MACH to control values in the TCES group, but it was without effect on MACH values in nonstimulated animals. TCES = transcranial electrical stimulation; NAL = naloxone; CTRL = controls.

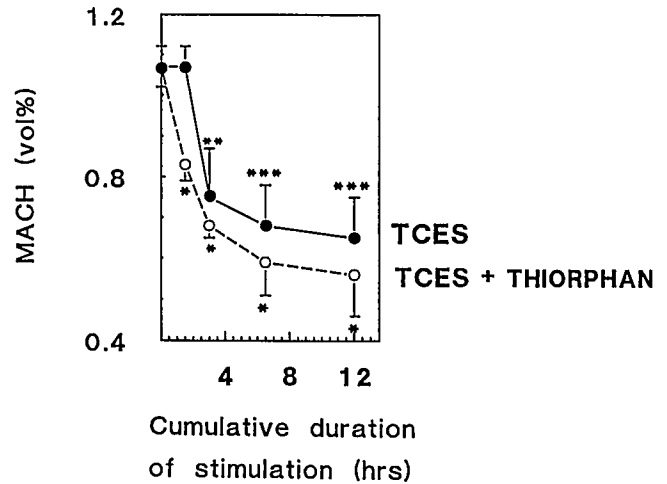


FIG. 3. Time course of TCES effects on MACH with (open circles) and without (filled circles) intracerebroventricular injection of a 5- μ g dose of thiorphan, which does not effect MACH in nonstimulated animals. No effect could be detected for periods of stimulation less than 2.8 h. A ceiling effect was observed for durations of stimulation exceeding 6.4 h. Thiorphan infusion resulted in significant potentiation of TCES effects for each duration of stimulation analyzed. * $P < 0.05$ versus thiorphan (Wilcoxon's paired test). ** $P < 0.01$ and *** $P < 0.001$ versus nonstimulated animals (ANOVA followed by Student's test).

< 0.001). No significant effect was observed following a 30-min period of stimulation. MACH values obtained after a 6.4-h period of stimulation, however, were not significantly different from those recorded using a 12.3-h stimulation. This indicates that both threshold (≈ 3 h) and ceiling (≈ 6 h) effects were present with respect to the cumulative duration of stimulation. For each duration of stimulation analyzed, a slight but significant potentiation of TCES effects was observed following infusion of an icv 5- μ g thiorphan dose (figure 3).

The results of the third set of experiments are displayed in figure 4. Intracerebroventricular infusion of naloxone (15 μ g) completely antagonized TCES effects on MACH, whereas neither yohimbine (30 μ g) nor methysergide (30 μ g) nor ritanserin (30 μ g) were effective in this regard. The 15-min delay used for equilibration following F_{IH} changes prohibited points for yohimbine, methysergide, or ritanserin corresponding to naloxone time points from being available.

Discussion

The present results indicate that TCES with Limoge's currents significantly decreases halothane requirements in rats. This phenomenon is very likely to be centrally mediated and occurs *via* enhancement of analgesia elicited by the release of endogenous opioids. In contrast, the α_2 -noradrenergic or the 5-HT₁ or 5-HT₂ serotonergic systems do not appear to be involved in this effect.

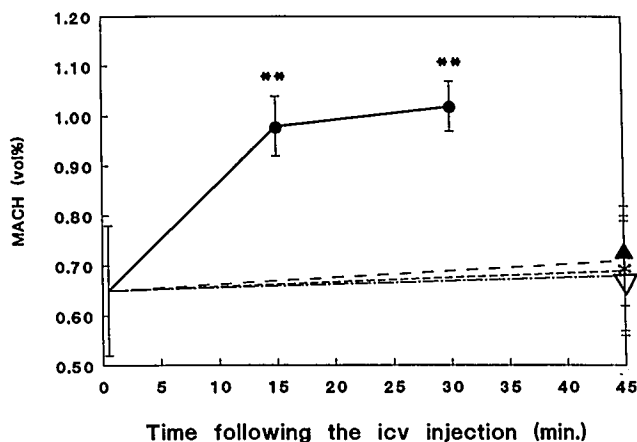


FIG. 4. Comparative effects of intracerebroventricular injections of naloxone (15 μ g, filled circles), yohimbine (30 μ g, open triangle), methysergide (30 μ g, large asterisk), and ritanserin (30 μ g, filled triangle) on the reversal of TCES effects on the MAC of halothane (MACH). All animals were stimulated for 10 h before tracheostomy. In contrast to naloxone, which rapidly restored MACH to the level of control values, the noradrenergic or serotonergic antagonists failed to affect the reduction of halothane requirements elicited by TCES. **A significant difference ($P < 0.01$, Wilcoxon's paired test) was present.

CHARACTERISTICS OF LIMOGÉ'S CURRENTS USED FOR TCES

Because analgesia produced by low transcranial electrostimulation depends on the electrical variables selected, TCES with high-frequency alternating balanced currents was developed in order to provide analgesia and, in addition, to circumvent the potential hazards related to electrical stimulation.²⁹ Mathematical analysis of Limogé's currents based on the Fourier series indicates that this complex pattern of stimulation allows deep penetration of the electric field into the brain without macroscopic damage in cerebral parenchyma.^{30,†} The dielectric properties of biologic tissue convert in situ the high-frequency current into a combination of a direct and a low-frequency current. The latter is believed to depolarize nerve terminals containing substances that participate in analgesic mechanisms. In fact, low-frequency modulated signals used for stimulation of the central gray matter in humans can achieve naloxone-reversible pain relief.⁸ Stimulation of this area by low-frequency currents very likely induces the release of endogenous opioids by depolarization of nerve endings, as assessed by the increased immunoreactivity for β -endorphins detected in the CSF of stimulated patients.⁹ Since endogenous opioids, and primarily enkephalins, are located in numerous brain areas, TCES

could cause their release from some brain structures (which remain to be determined precisely) and by inference could participate in opioid-mediated analgesic processes.

The intensity of the current used in our experiments was 100 mA peak to peak, which is much greater than the maximum intensity of a low-frequency current applied transcranially to a rat without inducing side effects.² The effective current passing through the brain could represent about 50% of the current applied to the head through anteroposterior surface electrodes.³¹ In the present study, the amperage of the effective current through rat brain was about one third that used in humans (52.5 vs. 17.6 mA).^{4,5} However, given the difference in size between rats' brains and humans' brains, the currents through any rat brain cell must have been far higher. It should be pointed out, however, that stimulating electrodes were applied directly to the cranial vault in rats, while cutaneous application was used in humans. Consequently, the currents delivered in rats would not have been diverted as much through skin and subcutaneous tissues. All of these interspecies differences could explain in part the discrepancy in onset of TCES effects between Stanley's *et al.*'s⁴ study performed in humans and ours. These differences also suggest that the results derived from this study using transcranial stimulation in rats may not be applicable to humans at all.

EFFECTS OF TCES ON HALOTHANE REQUIREMENTS

In the present study, TCES application was found to decrease markedly (-44%) the anesthetic requirements for halothane in rats. These data have been obtained using a randomized double-blind protocol, which rules out the possibility that the investigators might have been influenced during MACH determination. Moreover, the control MACH values found in our study were consistent with those obtained by others in rats.^{32,33} The standard deviation obtained for the MACH values in the stimulated animals was greater than in the controls, suggesting that the efficiency of TCES was not the same in all animals. This could be related to slight variations in the placement of the stimulating electrodes during animal preparation, which could have altered penetration of the electrical fields into brain tissue.

The magnitude of MACH reduction by TCES increased with the duration of stimulation until reaching a plateau from which no further decrease of MACH was observed. No effect was detectable for periods of stimulation shorter than about 3 h. This period of time represents the threshold of perceptibility for TCES effects. The absence of further potentiation of halothane anesthesia observed for periods of stimulation greater than 6 h may be due to a fading effect, which is classically re-

† Prieur G, Dubois F, Barrिताult L, Limogé A: Approche mathématique de l'action biologique des courants de Limogé. *J Biophys Méc* 9:67-74, 1985.

ported in electroanesthesia.³⁴ It can be suggested that fading was also responsible for the plateau phase observed in the potentiation by TCES of opioid-induced analgesia when durations of stimulation exceeded 3 h.^{3,35}

EVIDENCE FOR THE IMPLICATION OF THE ENDOGENOUS OPIOID SYSTEM IN TCES EFFECTS

The present study offers strong evidence that the reduction of halothane requirements elicited by TCES is mediated by the endogenous opioid system. In previous studies performed in rats, opiate-induced analgesia was found to be enhanced by TCES application.^{3,35} This phenomenon was suggested to be centrally mediated, since potentiation of analgesia was observed after icv infusion of narcotics. However, the choice of opiate-induced analgesia as an endpoint precluded the use of naloxone to test the reversal of TCES potentiation. The involvement of endogenous opioids in the mechanisms of general anesthesia has been debated in the past. Numerous groups have provided convincing evidence that the interaction between endogenous opioids and opioid receptors are not necessary for anesthetics to produce their anesthetic/analgesic effects.³⁶⁻³⁸ In agreement with these findings, we did not observe any significant difference in the mean MACH values between control animals and naloxone-treated rats. Therefore, the choice of MACH as an endpoint in the present study appears particularly appropriate to analyze whether the reduction of halothane requirements induced by TCES is mediated by an opiate-dependent mechanism.

In all rats tested, MACH reduction induced by TCES was reversed by the systemic delivery of a 2-mg/kg naloxone dose. These data are most supportive of the involvement of opiate receptors in TCES effects. The onset of naloxone action was rapid: all rats tested exhibited a positive response to the tail clamp as soon as the 15-min period, allowed to equilibration at the 1.1% F_{I_H} which followed naloxone administration, had ended. These findings are consistent with what could have been expected in relation to the pharmacokinetics and brain disposition of naloxone following systemic infusion of this compound in rats.²⁷ The effect of TCES on MACH was very likely to be centrally mediated since naloxone given icv was as effective as that administered systemically in antagonizing MACH reduction. It is interesting that analgesia produced by brain electrical stimulation has been shown to be reversed by systemic administration of naloxone in previous studies.^{8,39}

Our results strongly suggest that the opiate-dependent reduction of MACH in our study was associated with the potentiation of the analgesic action of enkephalins released by TCES into the extracellular space. Basically, a 5- μ g thiorphan dose given icv potentiated the effects of TCES for each duration of stimulation analyzed, leading

to a maximal MACH reduction of about 60% from control values. MACH has been shown previously to be decreased by as much as 80% following opioid administration in rats.⁴⁰ In our study, the effect of thiorphan was not additive to that of TCES itself, since thiorphan did not affect MACH in nonstimulated animals. The thiorphan dose chosen in this study was low: this could explain why the reduction of MACH by thiorphan delivered to TCES animals was moderate, even though statistically significant. As a matter of fact, thiorphan has been shown to enhance analgesia induced by enkephalins, and this effect was blocked by naloxone.^{29,41} Moreover, icv thiorphan administration combined with transcranial low-frequency low-intensity electrostimulation increases analgesia in an additive fashion.⁴² However, the analgesic doses of thiorphan reported in these studies are 4- to 50-fold greater than the 5 μ g dose.

Taken together, these data and ours strongly support the hypothesis that TCES might trigger the release of enkephalins from brain structures by a subtle mechanism that remains to be delineated. It can be speculated that the penetration of electrical fields into brain tissue induces the release of enkephalins by depolarizing enkephalin-containing neurons. These findings are consistent with the data of Yaksh and Chipkins who showed that in the spinal cord, thiorphan acts by potentiating the extracellular effects of enkephalins without affecting the tissue stores of this peptide.⁴³ This could explain why a 5- μ g thiorphan dose was inefficient in reducing MACH in nonstimulated animals. The release of enkephalins is certainly a good candidate to account for the potentiation of opiate-induced analgesia as well as the attenuation of morphine withdrawal syndrome by TCES.^{3,35} It is interesting that enkephalins are rapidly degraded not only by the neutral endopeptidase that cleaves either the met₄- or leu₅-enkephalin at the glycylphenylalanine amide bond, but also two aminopeptidases that either cleave the glycine-glycine bond or release the N-terminal tyrosine. Thus it can be speculated that other compounds blocking the activity of either the neutral endopeptidase or the aminopeptidase activities, such as SCH 34826, SCH 32615, or bestatin, could have the same effect as thiorphan in our model.^{41,43,44} However, it cannot be excluded that TCES elicits the release of other neuropeptides, such as endorphins, which have been shown to mediate naloxone-reversible analgesic effects.⁴⁵ Another possibility is that endogenous antiopiate systems such as the FMRF-amide system are inhibited by TCES.⁴⁶

FAILURE OF α_2 -NORADRENERGIC AND SEROTONERGIC ANTAGONISTS TO REVERSE THE TCES-INDUCED REDUCTION OF MACH

The reduction of MACH achieved by TCES was not affected by the icv infusion of either the α_2 NE antagonist

yohimbine or by the 5-HT antagonists methysergide (a nonselective blocker of the 5-HT₁ and 5-HT₂ receptors) or ritanserin (a selective 5-HT₂ antagonist). This is likely not to be due to the use of too-low doses of antagonists, since the doses used in the present study were found to be effective in specifically antagonizing spinal analgesia in previous studies performed in rats.^{18,25,27,28} However, the serotonergic system seems to be implicated in analgesia elicited by electrical stimulation with low-frequency currents.⁴⁷ Moreover, the 5-HT₃ receptors have been shown to mediate analgesia at the spinal level.⁴⁸ In the present study, drugs were injected icv and not intrathecally. However, there is some evidence that opiate infusion in upper brain structures can induce spinal analgesia that is mediated by spinal serotonergic and noradrenergic terminals.¹⁵ Therefore, it cannot be ruled out that some antagonism of TCES effects would have occurred if the NE and 5-HT antagonists were infused intrathecally. However, our data are consistent with those reported by Spanos *et al.*, who have shown that the antinociceptive action of two enkephalins at the spinal level were blocked by naloxone, but were affected neither by intrathecal administration of selective α_1 and α_2 NE antagonists nor by selective 5-HT₁, 5-HT₂, or 5-HT₃ antagonists.²⁸ Thus, it can be suggested that both the 5-HT and the α_2 NE systems were unlikely to be basically involved in the reduction of halothane requirements induced by TCES.

In conclusion, TCES with Limoge's currents markedly decreases the anesthetic requirements for halothane in rats. This effect appears to be related to the release of enkephalins from brain structures, thus enhancing opioid analgesia. The clinical relevance of these findings requires further investigation in humans.

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