Potentiation of ischemia-related behavioral alterations by electro-acupuncture in gerbils

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Summary

Gerbils subjected to global ischemia or sham-ischemia received electro-acupuncture (EA) or sham EA at points 26 Du (Renzhong) and 8 Du (Junsuo). All animals were then tested for motor activity in an open field, and for spontaneous alternation in a T maze. Results show that EA alone did not affect any behavioral parameter. Ischemia alone increased motor activity without significantly interfering with spontaneous alternation. EA in ischemic gerbils potentiated the increase of motor activity and elicited a decrease in spontaneous alternation. Thus, our data show an interaction between global ischemia and EA applied at specific acupoints which, however, consists of a potentiation rather than an alleviation of the behavioral alterations consecutive to the ischemic insult.

KEY WORDS: electro-acupuncture, functional recovery, global ischemia, motor activity, spontaneous alternation.

Introduction

Global transitory ischemia can produce permanent neurological alterations in animals as in man. In rodents, and particularly in gerbils, several areas are remarkably sensitive to ischemia with even brief occlusion of the carotid arteries being found to induce extensive cell loss in the CA1 hippocampal subfield, the dorsolateral striatum, the lateral septum, and layer 5 of the somatosensory cortex (1-3) that is apparent as soon as one day after the ischemic episode (4). As a consequence of this targeted neuronal damage, gerbils subjected to global ischemia show sensory motor deficits together with a number of behavioral alterations such as increased locomotor activity (4,5) and poor performance in hippocampal-dependent memory tasks (6-9). There is evidence that electro-acupuncture (EA) applied in various body points, identified by Chinese traditional medicine, can exert a neuroprotective effect or even enhance recovery of neurobiological alterations following experimental ischemia. For example, in the rat, EA applied at the late stage of the ischemic insult has been shown to restore the amplitude of somatosensory evoked potentials (10), the cortical levels of nitric oxide (NO) and endothelin (11), and the extracellular content of glutamate, aspartate and taurine in the striatum (12). EA has also been found to stimulate mechanisms involved in neuroprotection, such as the expression of basic fibroblast growth factor in astrocytes (13). However, to the best of our knowledge, no study has, until now, been conducted to examine whether EA is able to alleviate ischemia-related behavioral alterations. To investigate this possibility, gerbils with ischemia produced by bilateral occlusion of carotid arteries or sham-ischemia were subjected to EA or sham EA and then tested for locomotor activity in an open field (experiment 1) and for spontaneous alternation in a T maze (experiment 2).

Materials and methods

Forty male three-month-old gerbils purchased from the Charles River rearing center (Calco, CO, Italy) were used. They were housed in groups of 4 in a room with a 12h:12h light-dark cycle (lights on: 07.00-19.00) and food and water ad libitum. At the beginning of the experiments, they were approximately three months old and their weights ranged from 60 to 75 g. Surgery was performed under isofluorane anesthesia (3% during the induction phase and 1% during the maintenance phase) delivered in a mixture of 30% O2 and 70% NO2 by means of a Fluotec 3 vaporizer. The animals were placed in supine position and a midline incision of the cutaneous tissue was performed to expose the carotid arteries. Global ischemia (ISCH) was performed by clamping the carotid arteries with microaneurisimal clips for ten minutes, while the procedure for sham ischemia simply omitted the occlusion step. EA was administered at the same time as ischemia or sham ischemia in the
anesthetized animals. That is, the needles were inserted in the muscle at the intended acupoints (see diagrams published elsewhere showing the location of acupoints Du 26 (14) and Du 8 (15) in rodents) five minutes before the incision of the cutaneous tissue, then left in situ during surgery and for an additional period of 20 minutes. EA consisted of a total 30-minute electrostimulation (7 Hz, 5 mA). On each of the following seven days, the animals, non-anesthetized, were restrained in a plastic cage and subjected to EA, again for 30 minutes. The acupoints used were points Du 26 (Renzhong) and Du 8 (Junsuo). Traditionally, stimulation of point Du 26 (Renzhong) is used to enhance arterial blood pressure and heart rate in infarcted subjects (16), whereas stimulation of point Du 8 (Junsuo), frequently associated with stimulation of Du 16 (Feng Fu), has been reported to exert a neuroprotective effect notably by reducing the level of inducible NO synthase in ischemic animals (17). Sham EA was performed simply by restraining the animals in the cage used for delivering EA. Accordingly, the following groups were included in the experimental design: ISCH+EA, ISCH+sham EA, Sham ISCH+EA, Sham ISCH+Sham EA (control group). In addition, to avoid possible confounds resulting from aspecific factors, an additional group of ischemic subjects receiving EA in two non-acupoints located in the same area was considered (ISCH+pseudo EA). All groups had 8 subjects.

Behavioral testing started 24 hours after the last EA stimulation and was carried out during a single day. Five animals (one per condition) were subjected to behavioral testing on the same day. Experiments were conducted in compliance with the European Council directive (86/609/EEC) on the use and care of laboratory animals. Animals were first tested for motor activity in an open field (Auto Track System, Columbus Instruments, Columbus Ohio, USA), then for spontaneous alternation in a T maze. The open field consisted of a square cage (perimeter 40×4 cm) with a transparent floor. Infrared light sources situated on two opposite sides of the cage at two different heights (5 cm and 15 cm) from the floor allowed recording of various behavioral parameters, such as distance traveled, time spent resting, and number of rearing episodes. These behaviors were quantified through an interface (Auto Track) connected to a PC. The dependent variable collected was the distance (in cm) traveled in a period of five minutes. The spontaneous alternation phenomenon is the natural tendency of animals to explore alternately the right arm and the left arm of a T maze across successive runs, even in the absence of any reinforcing event (18,19). At the beginning of testing, animals were placed at the entrance of the starting arm (length: 40 cm) and allowed to explore the maze until they reached the end of the right (length: 25 cm) or the left (length: 25 cm) arm. Upon completion of every run, they were manually returned to the entrance of the starting arm. Each animal performed one trial consisting of nine consecutive runs. Four of the inter-run intervals lasted 5 seconds (short intervals) and the other four lasted 60 seconds (long intervals). The short and the long intervals were randomly distributed throughout the trial. The sequence of arm choices allowed us to calculate the alternation rate in each interval (short or long) condition.

Upon completion of the experiments, the gerbils were sacrificed by an overdose of chloral hydrate. The brains were fixed in formalin (10% solution) and cut in coronal sections of 40 µm using a cryostat. The sections were stained with cresyl violet according to the Nissl method.

Results

Histology

Figure 1 shows the neural tissue in a control (sham ISCH+sham EA) gerbil (A) and in gerbils subjected to global ischemia and EA. In general, ischemia produced extensive bilateral loss of pyramidal cells in the CA1 hippocampal subfield (C), although some animals exhibited only unilateral damage (B). The proportion of bilateral and unilateral damage was similar in all the groups of gerbils subjected to ischemia. Light microscopy examination of the neural tissue did not reveal any difference between ischemic groups receiving EA, sham EA or pseudo EA.

Figure 1 - Representative coronal sections stained with cresyl violet showing cell loss in the CA1 subfield of the hippocampus in gerbils subjected to global ischemia and electro-acupuncture at points Du 26 (Renzhong) and Du 8 (Junsuo) (top) and in gerbils subjected to ischemia and sham electro-acupuncture (middle) at the same acupoints. No cell loss is evident in gerbils subjected to sham ischemia and sham electro-acupuncture (bottom).
Behavior

Motor activity scores (Fig. 2) were analyzed by a one-way ANOVA (group factor, five levels). Alternation scores (Fig. 3) were analyzed by a two-way ANOVA with group (five levels) and delay (two levels) as main factors. Simple effects and pair comparisons (Newman-Keuls test) were calculated where necessary. The ANOVA performed on the motor activity data revealed a significant effect of the group factor [F(1,4) = 7.20, p<.001] indicating that the distance traveled varied according to the treatment received. Subsequent pair comparisons revealed that motor activity was significantly higher in the ischemic gerbils subjected to EA than in the four other groups. Ischemia alone enhanced locomotor activity (p<.05) relative to the control group. Interestingly, the scores of gerbils subjected to ischemia followed either by sham EA or pseudo EA (that is, EA either not administered or administered in a non acupoint), were not statistically different (p=.9) thus ruling out an aspecific effect of the electrical stimulation on motor activity scores. Finally, EA was not found to increase motor activity in non ischemic gerbils. It is therefore apparent that EA delivered at the acupoints considered potentiates the increase of motor activity induced by ischemia, but does not affect motor activity in non ischemic gerbils. The ANOVA performed on the spontaneous alternation data indicated a significant group x delay interaction [F (4,29)=3.89, p<.02]. Alternation scores in the short (5 s) delay condition were generally high in all the groups except ISCH+EA, that is, a significant effect of the “group factor” was found for the 5 s delay [F (4,29)=3.55, p<.02] with pair comparisons indicating that gerbils subjected to both ischemia and EA alternated significantly less than the other groups (p<.05 for all pair comparisons). This observation might suggest that the combination of the two treatments produces a disruptive effect on working memory that does not emerge following each single treatment. However, this hypothesis of a working memory deficit in the ISCH+EA group was challenged by the consideration that these gerbils, which showed enhanced motor activity, could simply be impaired in controlling their behavior at the choice point of the T maze. In fact, two observations are consistent with the latter assumption. First, no main effect of the “group factor” was found in the longer delay condition [F (4,29)=0.48, p=.74]. That is, all gerbils were now displaying similar alternation scores, whereas a possible working memory deficit in the ISCH+EA group should have been more pronounced in the long delay condition. Second, there was no significant main effect of the delay factor, [F(1,29)=0.7, p=.4]. Thus, a longer delay interposed between runs did not significantly decrease spontaneous alternation in any group but just restored the alternation scores of the ISCH+EA group to a level similar to that recorded in the other groups.

Figure 2 - Mean distance (cm) traveled (+ S.E.) during a five minute session in the open field by gerbils subjected to global ischemia or sham ischemia, and electro-acupuncture or sham or pseudo electro-acupuncture delivered at points Du 26 (Renzhong) and Du 8 (Junsuo). § different from all other groups (p<.05). * p<.05.

Figure 3 - Percentage of spontaneous alternation in the T maze following 5 s (top) or 60 s (bottom) inter-run intervals in gerbils subjected to global ischemia or sham ischemia, and electro-acupuncture or sham or pseudo electro-acupuncture delivered at points Du 26 (Renzhong) and Du 8 (Junsuo).
Discussion

Taken together, these data allow the following points to be made. Ischemia alone increased motor activity whereas EA alone did not. The first observation is consistent with data from the literature showing that global ischemia, because of the hippocampal damage produced by the carotid occlusion, increases motor activity as other unilateral (20) or bilateral hippocampal lesions do (21). The second observation, on the other hand, seems to conflict with previous data showing an increase in motor activity following EA in the rat (22). In this latter experiment, however, EA was applied at a different acupoint (Bai Hui point) and was found specifically to activate central monoamine neurotransmission. Thus, the main result of the present experiments is that EA administered at the 26 Du (Renzhong) and 8 Du (Junsuo) acupoints does not reduce but potentiates the increase of motor activity produced by ischemia, even though examination of the neural tissue does not reveal a different extent of cell loss in ischemic gerbils subjected to EA vs those receiving sham EA. Clearly, the absence of increased morphological damage in ischemic gerbils subjected to EA could be explained by the fact that gerbils were sacrificed shortly after behavioral testing thus preventing long-term effects of EA-related functional brain modifications – such as NO synthase synthesis – on brain morphology from being observed. The spontaneous alternation data also show that gerbils subjected to both ischemia and EA poorly alternate under the short delay condition. This finding, however, which might point to an impairment of working memory in the ISCH+EA group, is likely to depend on the high level of motor activity recorded in this group. Indeed, the absence of a significant effect of ischemia alone on spontaneous alternation is intriguing. However, it is worth noting that spontaneous alternation deficits following ischemia have often been observed in gerbils, both tested in more complex alternation tasks [e.g., reinforced alternation, longer inter-run intervals, (23)] and subjected to repeated occlusion episodes (24).

A possible explanation of the negative effect of EA on the behavior of ischemic gerbils relates to the physiological effects induced by stimulation of the 26 Du (Renzhong)–EA group, is likely to depend on the high level of motor activity recorded in this group. Indeed, the absence of a significant effect of ischemia alone on spontaneous alternation is intriguing. However, it is worth noting that spontaneous alternation deficits following ischemia have often been observed in gerbils, both tested in more complex alternation tasks [e.g., reinforced alternation, longer inter-run intervals, (23)] and subjected to repeated occlusion episodes (24).

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References

2. Levine S, Sohn D. Cerebral ischemia in infant and adult gerbils. Relation to incomplete circle of Willis. Arch Pathol 1969;87:315-317
14. Wang SJ, Omoi N, Li F et al. Enhanced expression of...


