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TOLERANCE TO ELECTROACUPUNCTURE AND ITS CROSS TOLERANCE TO MORPHINE

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Summary—Electroacupuncture (EA) applied to both legs of the rat for 30 min (1 session) raised the average tail flick latency to 89% above the control level. Repeated electroacupuncture for 6 sessions, with 30 min between successive sessions, resulted in a gradual decline in the hypoalgesic effect. The time-course of the development and disappearance of acupuncture tolerance was studied. A bi-directional cross-tolerance between electroacupuncture and morphine points to the similarity between the underlying mechanisms of electroacupuncture hypoalgesia and morphine analgesia.

Repeated administration of morphine causes a gradual diminution of the analgesic effect. This change is termed morphine tolerance. Similarly, repeated or prolonged application of electroacupuncture (EA) to rats resulted in a gradual fading away of the hypoalgesic effect; this has been termed "acupuncture tolerance" (Tang, Liang, Zhang and Han, 1979; Ren and Han, 1979). A comparable situation has been described for the stimulation-produced analgesia (SPA) (Mayer and Hayes, 1975; Hosobuchi, Adams and Linchitz, 1977) and such a tolerance turns out to be one of the limiting factors which curtails the effective use of stimulation-produced analgesia in clinical practice. In this paper, the time-course of the development and disappearance of acupuncture tolerance (AT) and its relationship to morphine tolerance (MT) is described.

METHODS

Acupuncture tolerance

Female rats weighing 180–200 g were used with electroacupuncture being performed as previously described (Ren and Han, 1979). Rats were partially restrained in specially designed holders with the hind legs and tail protruding outside the holder. Two pairs of stainless-steel needles were inserted in both legs. Stimuli of 2–15 Hz biphasic electric pulses of 0.3 msec duration were applied using a 57-6D electronic stimulator. Starting from 1 V, the amplitude of the negative pulse was increased by 1 V every 10 min and reached 3 V as maximal value. One session of electroacupuncture lasted for 30 min. Such a stimulation period was repeated after a 30 min interval.

The nociceptive response was measured by the tail flick latency elicited by radiant heat (Ren and Han, 1979). The basal level of tail flick latency was obtained by averaging the two measurements before the application of electroacupuncture. The measurement was then repeated every 10 min after the start of the electrical stimulation and the values expressed as a percentage change of the basal level. The average value of the 3 measurements during electroacupuncture was assigned as the average effect.

Morphine tolerance

To test the effectiveness of morphine analgesia, 6 mg/kg of morphine HCl was injected subcutaneously and the tail flick latency measured before, and every 10 min after, the drug administration. The percentage change of 5 postdrug measurements were averaged and taken as the average morphine effect. To induce morphine tolerance, rats were injected subcutaneously 3 times a day for 8 days with increasing doses of morphine HCl from 5 mg/kg up to 50 mg/kg (5, 10, 15, 20, 25, 30, 40 and 50 mg/kg respectively). Sensitivity to morphine analgesia was monitored at various times to assess the onset and reversal of morphine tolerance. In order to test cross tolerance between acupuncture tolerance and morphine tolerance, rats tolerant to morphine were given electroacupuncture and rats with acupuncture tolerance were given a testing dose of morphine (6 mg/kg). In the later case, only one morphine injection was allowed for each rat.

Chemicals

Morphine HCl was obtained from Shenyang Drug Company and naloxone HCl was purchased from Endo Laboratories.

Statistics

Student's *t*-test was used for statistical analysis. The regression line in the correlation analysis was fitted by the least square method.

RESULTS

Time-course of the development and disappearance of acupuncture tolerance

Six sessions of electroacupuncture were given to a group of 30 rats. The averaged effect on tail flick

Key words: acupuncture, electroacupuncture, morphine analgesia, morphine tolerance

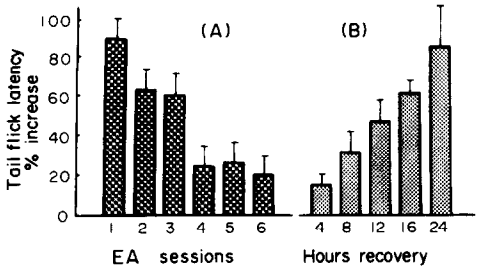

Fig. 1. The time course of development (A) and disappearance (B) of electroacupuncture tolerance. Each session of acupuncture lasted for 30 min. The columns represent the percentage increase in tail flick latency which was the average value of 3 assessments in 30 min. Vertical lines indicate the SEM.

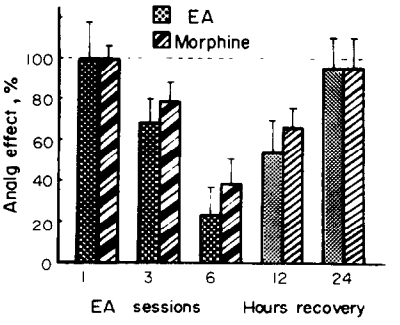

Fig. 2. Changes in the effectiveness of morphine analgesia following the development of tolerance to acupuncture. The increase of tail flick latency by the first acupuncture or morphine treatment was taken as 100%.

latency is shown in Figure 1(A). In the first session there was an increase in tail flick latency of 89 ± 12% (mean ± SEM). The intensity of the response decreased gradually with successive electroacupuncture sessions to 61 ± 11% (3rd EA) and 21 ± 9% (6th EA).

The time-course of recovery from acupuncture tolerance was tested in 60 rats. These animals were rendered tolerant to electroacupuncture with the procedure stated above, and divided into 5 groups. Each group was then given one session of electroacupuncture 4, 8, 12, 16 or 24 hr respectively after the last session. The results are shown in Figure 1(B). The degree of acupuncture tolerance remained unchanged for at least 4 hr. After this time interval, the acupuncture tolerance decreased stepwise and the hypoalgesic action of electroacupuncture approached the original level after 24 hr; i.e. acupuncture tolerance which had developed during 6 hr of stimulation was completely obliterated in 24 hr.

To rule out the possibility that the gradual decline of the electroacupuncture effect was the result of prolonged restraint, a group of 6 rats was restrained in the holder as stated above. Then, electroacupuncture was given after various periods of restraint. The averaged effect was found to be 108 ± 17, 115 ± 17 and 110 ± 13% after 0, 6 and 30 hr of restraint respectively, showing no significant tendency to diminish.

To rule out the possibility of local tissue damage, two needles, A and B, were placed 5 mm apart into each point area. Stimulation was applied through device A and B to test their respective ability to induce the hypoalgesic effect. It was found that they elicited an increase in tail flick threshold which was 116 ± 10 for A and 108 ± 8% for B, respectively. This value was taken as 100%. Repeated electroacupuncture for 6 sessions with device A resulted in a decrease of tail flick latency to 7 ± 8% of the original response. If this decrease was a result of the polarization of the needle or the damage to tissue immediately adjacent to the needles, the effect would have reappeared when device B was used for stimulation. A very low shift in tail flick latency was found when device B was used (24 ± 12%, n = 8). This value was not significantly different from the effect elicited by stimulation through device A.

Changes in the effectiveness of morphine analgesia following the development of acupuncture tolerance

Fifty rats were divided into 5 groups of 9–11 animals each. Each group was injected with a test dose (6 mg/kg) of morphine HCl at the end of the 1st, 3rd or 6th session of electroacupuncture as well as 12 or 24 hr after the 6th session, respectively. The results are shown in Figure 2. Taking the increase in tail flick latency by the first electroacupuncture or morphine treatment as 100%, the effect of electroacupuncture in the 6th session was found to be only 24 ± 13% and that of morphine 39 ± 13% of the original value.

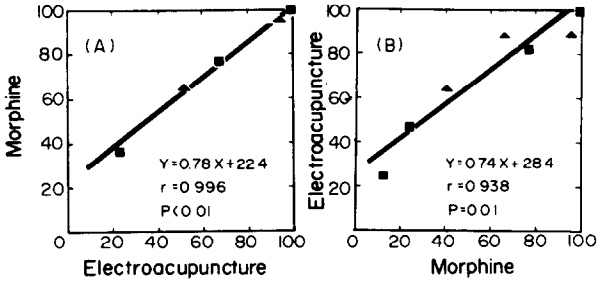

Fig. 3. Cross tolerance between electroacupuncture hypoalgesia and morphine analgesia. (A) The data from Figure 2 was analyzed. ■ represent the course of development of acupuncture tolerance, ▲ the course of its disappearance. Each point corresponds to 9–11 rats. (B) The data from Figure 4 was analyzed. ■ represent the course of development of morphine tolerance, ▲ the course of its disappearance. Each point corresponds to 10 rats. Equations for the regression lines are shown in the Figures.

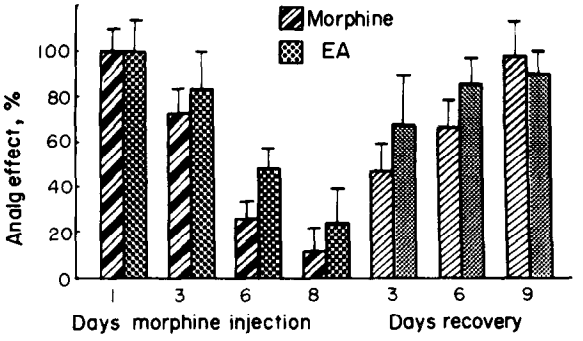

Fig. 4. Changes in the effectiveness of electroacupuncture hypoalgesia following the development of tolerance to morphine. The increase of tail flick latency by the first acupuncture or morphine treatment was taken as 100%.

After the cessation of repeated electroacupuncture, the effect of morphine recovered gradually, approaching the basal level (96 ± 13%) in 24 hr.

In Figure 3(A), the data of above 5 groups are plotted with a percentage change in averaged electroacupuncture effect as abscissa (X) and that of averaged morphine analgesia as ordinate (Y). A positive correlation between the two parameters ($r = 0.996$) pointed strongly to the existence of cross tolerance.

Changes in the effectiveness of electroacupuncture following the development of morphine tolerance

A group of 10 rats was given an increasing dose of morphine for 8 days. A testing dose of morphine was administered to assess its potential analgesic effect during days 1, 3, 6, 8 and 3, 6 and 9 days after chronic morphine treatment. The results are shown in Figure 4. Taking the averaged morphine effect on day 1 as 100%, the values on day 6 and 8 were only 25 ± 10 and 12 ± 10% ($P < 0.001$ as compared to day 1). After cessation of chronic morphine treatment, the analgesic effect of morphine reappeared and its intensity approached a normal level (98 ± 14%) in 9 days.

The effectiveness of electroacupuncture was also tested on the same experimental day as that for assessment of morphine analgesia. It can be seen from Figure 4 that there is a parallel change between the two indices. On day 6 and 8, the averaged electroacupuncture effect was found to be only 48 ± 9 and 25 ± 15% ($P < 0.001$) that of the control value. After the cessation of morphine administration, the increase in tail flick latency elicited by electroacupuncture recovered stepwise, approaching the normal level in 9 days.

The same set of data in Figure 4 are replotted in Figure 3(B) with the percentage change in morphine analgesia as abscissa and that of electroacupuncture as ordinate. They fall in a regression line with the correlation coefficient r = 0.938 ($P < 0.01$), suggesting a positive correlation between acupuncture tolerance and morphine tolerance.

Naloxone precipitated withdrawal syndrome

Intraventricular injection (Noble, Wurtman and Axelrod, 1967) of 8 μg/20 μl of naloxone to a group of 6 morphine-tolerant rats (8 days schedule) elicited prominant signs of abstinence including irritation and attempts to escape, salivation, diarrhea and a decrease of rectal temperature from 37.6 ± 0.24 to 35.5 ± 0.17°C (−2.1°C, $P < 0.01$). In a group of 6 rats rendered tolerant to electroacupuncture by 6 sessions of stimulation, the same dose of naloxone (intraventricular) induced no sign of abstinence during a 1 hr observation period except for one rat showing a moderate degree of irritability. The decrease in rectal temperature from 37.7 ± 0.23 to 37.2 ± 0.26°C (−0.5°C) was statistically not significant. The results indicated that while prolonged electroacupuncture caused the development of tolerance to acupuncture and morphine, no naloxone-precipitated withdrawal syndrome was observed in acupuncture-tolerant rats.

DISCUSSION

It has been demonstrated that during acupuncture hypoalgesia there is an increase in the content of endogenous opiate-like substances in the brain (Tang and Han, 1978; Zou, Yi, Wang, Lu, Zhang and Wu, 1979) and cerebrospinal fluid (Sjolund, Terenius and Erikson, 1977). It is also a well-established fact that repeated injection of opiate-like substances into cerebroventricles will elicit tolerance as well as cross tolerance to morphine (van Ree, de Wied, Bradbury, Hulme, Smyth and Snell, 1976). It is thus reasonable to postulate that repeated or prolonged electroacupuncture which releases in the brain a large amount of opiate-like substances (Acupuncture anesthesia research group, Shanghai First Medical College, 1978a) may cause a gradual decrease in its hypoalgesic effect which can be termed acupuncture tolerance. The evidence provided in this paper clearly demonstrates the existence of acupuncture tolerance, and the time course of its development and disappearance. The bi-directional cross tolerance of electroacupuncture with morphine and their fairly well correlated temporal coincidence gave additional support to the proposition that acupuncture tolerance is related to a tolerance to opioids, and points to the similarity between the mechanisms underlying acupuncture and morphine tolerance. There are, however, some minor discrepancies which point to the difference between the primary tolerance and the induced cross-tolerance. From the regression lines shown in Figure 3(A) and (B) it is evident that both slopes are less than unity (0.78, 0.74) and both of them possess considerable intercepts (22.4, 28.4), which suggests that when the effect of electroacupuncture in the acupunture tolerant rats approaches zero, the effect of morphine still remains 1/4 of its original value and vice versa. Hence, one might infer that the underlying mechanisms for acupuncture hypoalgesia and morphine analgesia are not identical. This is in agreement with the notion that acupuncture not only activates the release of opioids from CNS stores but also activates a host

of other synaptic mechanisms among which only a few have been identified (Han, Tang, Ren, Zhou, Fan and Qiu, 1981).

There are some controversial reports concerning the effectiveness of acupuncture hypoalgesia in morphine-tolerant animals. Thus, one research group in China (Acupuncture Anesthesia Research Group, Shanghai First Medical College, 1978b) found no suppression of acupuncture analgesia in rabbits made tolerant to morphine by continuous intravenous morphine infusion for 8 hr (total dose 40–50 mg/kg). Another group in Canada (Cheng, Pomeranz and Yu, 1980) found an enhancement of acupuncture hypoalgesia in morphine-dependent mice. In view of the fact that development of tolerance to morphine or acupuncture is a dynamic process (see Figs 1, 2 and 4), it is quite conceivable that the phenomenom of cross tolerance can be fully demonstrated only when precise point is reached in morphine tolerance. Though it is worth keeping in mind variations due to species difference it may be mentioned here that, in this laboratory, cross tolerance to acupuncture and morphine has been shown to occur in rabbits when a more intensive schedule was used to induce morphine tolerance (unpublished data).

It is a common characteristic of narcotics that tolerance is always accompanied by physical dependence (Way, Loh and Shen, 1969). Whereas repeated electroacupuncture elicits acupuncture tolerance, it does not infer that the abrupt cessation of acupuncture or the administration of a narcotic antagonist in tolerant rats would induce withdrawal symptoms or precipitate an abstinence syndrome. It is plausible that the heterogenecity of the opiate receptor pool mediating various actions of narcotics and opioids can explain this difference. From a series of behavior-related studies Jacquet (1978) suggested that there are two classes of opiate receptors in the peri-aqueductal grey (PAG); one is naloxone-sensitive, mediates analgesia and develops tolerance, its endogenous ligand being β-endorphin: the other is naloxone-insensitive, causes explosive motor behavior, its endogenous ligand being ACTH. If acupuncture elicited a selective release of β-endorphin in peri-aqueductal grey, then the abrupt cessation of acupuncture or the administration of naloxone would not have resulted in the precipitation of an abstinence syndrome. However, evidence provided by Wei and Loh (1976), in rats, indicated that physical dependence developed on the continuous administration of met^5-enkephalin or β-endorphin into the peri-aqueductal grey for 70 hr and typical signs of abstinence appeared when challenged with naloxone. At the present time the simplest explanation for tolerance to, without dependence on acupuncture seems to be the insufficient amount of opioids released during acupuncture for inducing dependence. Further investigation is needed for clarification of this problem.

In conclusion, data has been presented showing that repeated electroacupuncture in rats resulted in the development of tolerance to, without dependence on acupuncture. The cross tolerance between acupuncture and morphine indicates a similar, although not identical, underlying mechanism between acupuncture hypoalgesia and morphine analgesia.

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