Analgesia induced by electroacupuncture of different frequencies is mediated by different types of opioid receptors: another cross-tolerance study

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The cross-tolerance technique was used to analyze the receptor mechanisms of analgesia induced by electroacupuncture (EA) of 2 Hz, 100 Hz, or 2–15 Hz. (1) Rats were given EA stimulation of 2 Hz, 100 Hz and 2–15 Hz for 30 min with 30 min intervals successively. The percentage increase in tail-flick latency (TFL) was taken to indicate the intensity of EA analgesia. Rats made tolerant to repeated intrathecal injection of the μ-opioid agonist ohmefentanyl (OMF, 15 pmol, Q2h × 5) or the δ-opioid agonist DPDPE (10 nmol, Q2h × 5) showed a cross tolerance to both 2 Hz- and 2–15 Hz-, but not to 100 Hz-EA analgesia; and rats made tolerant to κ-opioid agonist dynorphin-(1-13) (5 nmol, Q2h × 5) showed a cross-tolerance to 100 Hz- and 2-15 Hz-, but not to 2 Hz-EA analgesia; (2) Rats made tolerant to 2-15 Hz EA showed cross-tolerance to either 2 Hz- or 100 Hz-EA analgesia; (3) Rats made tolerant to either 2 Hz- or 100 Hz-EA were still reactive to 2–15 Hz-EA. The results indicate that 2 Hz-EA analgesia is mediated by μ- and δ-receptors, 100 Hz-EA analgesia by κ-receptor, and 2–15 Hz-EA analgesia by combined action of μ-, δ- and κ-receptors in the spinal cord of the rats.

INTRODUCTION

Our previous studies suggest that 2 Hz- (low frequency) electroacupuncture (EA) analgesia is mediated by met-enkephalin via δ-receptors, and 100 Hz- (high frequency) EA analgesia by dynorphin via κ-receptors in the spinal cord of the rat[3,4,7,19,21]; whereas analgesia induced by 2–15 Hz- (dense-and-disperse mode) EA is mediated by both met-enkephalin and dynorphin[7] via the simultaneous activation of μ-, δ-, κ-receptors in the spinal cord of the rat (to be published). A cross-tolerance study is a powerful technique commonly used in pharmacology studies to characterize whether two kinds of drugs are acting on the same type of receptor. The cross-tolerance technique can be used in two directions: (A) in rats made tolerant to EA analgesia, we tested their response to an exogenously applied receptor agonist; (B) in rats made tolerant to a specific receptor agonist, we tested their response to EA stimulation. We

have shown previously that rats made tolerant to 2 Hz-EA analgesia showed cross-tolerance to DPDPE-induced analgesia but not to dynorphin-induced analgesia; and that rats rendered tolerant to 100 Hz-EA showed a diminished response to dynorphin-(1-13), yet the analgesic effect induced by DPDPE remained intact[19]; and that rats made tolerant to 2–15 Hz-EA analgesia were cross tolerant to all three type of opioid agonists, OMF, DPDPE and dynorphin-(1-13) (to be published). These results suggest that 2 Hz-EA analgesia is mediated by the δ-receptor, 100 Hz-EA analgesia by the κ-receptor, and 2–15 Hz-EA analgesia by the combined activation of μ-, δ- and κ-receptors. Deprivation of any one of the three opioid receptors would impair the whole system subserving 2–15 Hz-EA analgesia in the spinal cord of the rat.

In the present study, we have used approach B. Highly specific opioid ligands, ohmefentanyl (OMF) for the μ-receptor[6,12,23,25], DPDPE for the δ-receptor[17] and dynorphin A for the κ-receptor[22], were used as pharmacological tools for analyzing the receptor mechanisms underlying 2 Hz-, 100 Hz- and 2–15 Hz-EA analgesia.

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MATERIALS AND METHODS

Experiments were performed in adult Wistar rats weighing 200–300 g. Under chloral hydrate (400 mg/kg, i.p.) anesthesia, a midline skin incision was made over the back of the neck. The atlanto-occipital membrane was exposed and carefully incised for insertion of a polyethylene tubing (PE-10, outer diameter 0.61 mm) into the subarachnoid space to reach the lumbar enlargement of the cord. The catheter was threaded caudally 7.5 cm to the lumbar spinal cord and fixed in situ with 5 cm of the tubing left outside the closed wound[24]. Experiments began 24 h after the operation. Only rats with completely normal motor function were used. The volume of intrathecal (i.t.) injection was 10 μl followed by 10 μl of normal saline for flushing. The whole procedure of injection was completed in 1 min.

Ohmefentanyl (OMF), N-[1-(2-hydroxy-2-phenyl-ethyl)-3-methyl-4-piperidyl]-N-phenylpropionamide was produced by the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai; dynorphin-(1-13) was a gift of Professor Avram Goldstein, Addiction Research Foundation, Palo Alto, U.S.A.; [D-pen^2,D-pen^5]enkephalin (DPDPE) was a product of Sigma.

The nociceptive response was measured by the tail-flick latency (TFL) elicited by radiant heat[18]. Rats were partially restrained in plastic holders with the hind legs and the tail protruding. Focused light from a 12.5-W projection bulb was applied to an area located between the middle and the lower $^1/_3$ of the tail of the rat and the TFL was recorded by an electronic timer with room temperature maintained at 20 $\pm$ 1 °C. At the beginning of the experiment, 3 TFL assessments were performed with 5 min apart, and the mean value was taken as the basal pain threshold, usually within the range of 4–6 s. The values of the subsequent measurements after EA or drug administration were expressed as percentage change from the baseline level. Two preventive measures were taken to avoid unnecessary burning of the skin: (1) a cutoff limit of + 150% was set, and (2) the radiant heat was focused on the skin within an area of 1 cm long rather than on a fixed spot.

Electroacupuncture (EA) analgesia and EA tolerance experiments were performed as follows. Two pairs of stainless-steel needles of 0.25 mm diameter were inserted in both legs at the acupoint Zusanli (S36, 5 mm lateral to the anterior tubercle of tibia) and Sanyingjiao (SP6, 3 mm proximal to the medial malleolus, at the posterior border of the tibia). Five min after the last measurement of the basal pain threshold, bidirectional square waves of 0.3 ms duration from a programmed pulse generator (HGC-24, made in China) were applied on the needles in each leg. The frequency of the square waves can be set in 2 modes: the continuous mode delivers constant frequency stimulation of 2 Hz or 100 Hz, whereas in the dense-and-disperse (D-D) mode 2 Hz was alternating with 15 Hz automatically (2–15 Hz), each lasting for 2.5 s[8]. In the experiment for EA analgesia, the intensity of stimulation (constant current) was set at 1 mA at the beginning of the experiment and increased by 1 mA every 10 min to reach 3 mA as maximal level. In the EA tolerance experiment, EA stimulation was given continuously for 6 h, with a short break at the end of each hour for the measurement of the TFL. In the latter case the analgesic effect of EA was found to decrease gradually and approach zero at the end of the 6-h stimulation period, showing the development of tolerance to EA.

Tolerance to intrathecally injected opioid agonists was observed as follows. Intrathecal injection of opioid agonist (μ-agonist OMF 15 pmol, δ-agonist DPDPE 10 nmol, or κ-agonist dynorphin-(1-13) 5 nmol) was given every 2 h for a total of 5 injections. The TFL was measured before the injection and every 10 min after the injection. The mean value of the 3 post-injection assessments with 10 min apart was taken and expressed as percentage change of the baseline level, which was taken as an index of the opioid analgesia. The gradual decrease of the analgesic effect after repeated i.t. administration indicated the development of drug tolerance.

The time table of the experiments was as follows. In the agonist tolerance experiment, rats were given successive EA stimulation of 2 Hz, 100 Hz and 2–15 Hz, each lasting for 30 min with half-hour intervals. Thirty min after the last session of EA, the rats were given i.t. injections of either the μ-agonist OMF, δ-agonist DPDPE or κ-agonist dynorphin-(1-13) every 2 h for a total of 5 injections. Two hours after the last injection, rats were again given 2 Hz-, 100 Hz- and 2–15 Hz-EA, respectively, with 30 min intervals. In the EA tolerance experiment, rats were given 30 min testing EA stimulation of identified frequency, followed 30 min later by EA of another frequency given continuously for 6 h which was interrupted only by hourly TFL assessments. At the end of the 6-h stimulation period, the 30 min testing EA was repeated.

Data are expressed as the mean and its standard error. Statistical analysis of difference between groups was assessed with paired t-test. $P < 0.05$ was taken as significant level of difference.

RESULTS

Tolerance to ohmefentanyl (OMF)-produced analgesia and cross-tolerance to EA analgesia

A group of 8 animals were given EA of 2 Hz, 100 Hz and 2–15 Hz successively as stated in methods. The stimulations produced increases in TFL of $102 \pm 9\%$, $84 \pm 11\%$ and $126 \pm 11\%$, respectively. Rats were then given i.t. injection of the μ-agonist OMF 15 pmol, and TFL was measured every 10 min for 30 min to obtain an average analgesic effect. The i.t. injection was repeated every 2 h for 5 times. The results are shown in Fig. 1. The analgesic effect of OMF decreased gradually and disappeared at the end of the fifth session. Two hours after the fifth OMF injection, rats were again given EA of 2 Hz, 100 Hz and 2–15 Hz as mentioned above. The percentage changes of TFL after 2 Hz, 100 Hz and 2–15 Hz EA were $3 \pm 2\%$ (paired t-test, $P < 0.01$ as compared to $102 \pm 9\%$), $75 \pm 9\%$ ($P > 0.05$ as compared to $84 \pm 11\%$) and $6 \pm 2\%$ ($P < 0.01$ as compared to $126 \pm 11\%$) respectively. The results suggested a cross-tolerance between OMF produced analgesia and the analgesia induced by EA of 2 Hz or 2–15 Hz. In contrast, the analgesia induced by 100 Hz EA was not significantly affected.
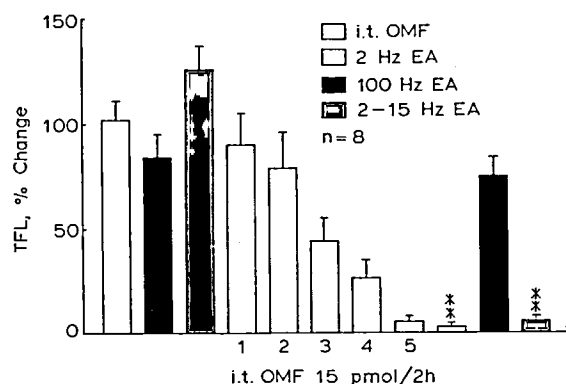

Fig. 1. Tolerance to ohmefentanyl (OMF)-produced analgesia and cross-tolerance to analgesia induced by electroacupuncture (EA) of different frequencies. Rats were given EA of 2 Hz (white columns), 100 Hz (black columns) or 2–15 Hz (dark grey columns) for 30 min each, and the percentage of increase in tail-flick latency (TFL) was measured at the end of 30 min as an index of EA analgesia. They were then given an intrathecal (i.t.) injection of OMF 15 pmol (light grey columns), and TFL measured every 10 min for 30 min. The average of 3 measurements were taken as the index of OMF-produced analgesia. The injection was given every 2 h (Q2h) for 5 times. Two hours after the last injection, the effectiveness of EA analgesia was tested again in the same procedure as for the pre-injection trials. **$P < 0.01$ as compared to the corresponding value taken prior to drug administration by the paired t-test. n represents the number of animals.

Tolerance to DPDPE-produced analgesia and cross-tolerance to EA analgesia

A group of 8 rats were given successive EA stimulation of 2 Hz, 100 Hz and 2–15 Hz, each lasting for 30 min with 30 min intervals. The analgesic effects were $121 \pm 12\%$, $126 \pm 11\%$ and $132 \pm 7\%$, respectively. The rats were then given i.t. injections of the δ-agonist DPDPE 10 nmol in a similar schedule as mentioned in the preceding study for OMF. The results are shown in Fig. 2. The analgesic effect of DPDPE decreased gradually from $125 \pm 8\%$, $89 \pm 11\%$, $48 \pm 8\%$, $22 \pm 7\%$ to $4 \pm 2\%$ at the end of the fifth injection, suggesting the development of DPDPE tolerance. Rats were then given 2 Hz-, 100 Hz- and 2–15 Hz-EA respectively. The percentage change of TFL were $5 \pm 2\%$ (paired t-test, $P < 0.01$ as compared to $121 \pm 12\%$), $110 \pm 10\%$ ($P > 0.05$ as compared to $126 \pm 11\%$) and $7 \pm 4\%$ ($P < 0.01$ as compared to $132 \pm 7\%$), respectively, indicating that rats rendered tolerant to δ-agonist DPDPE showed complete cross-tolerance to either 2 Hz- or 2–15 Hz-EA, but not to 100 Hz-EA-induced analgesia.

Tolerance to κ-agonist dynorphin-(1-13)-produced analgesia and cross-tolerance to EA analgesia

The protocol was the same as in the previous experiment except that the κ-agonist dynorphin-(1-13) was used instead of DPDPE. Rats ($n = 9$) were given EA stimulation of 2 Hz, 100 Hz and 2–15 Hz for 30 min each, and the tail flick latency was found to increase by $135 \pm 7\%$, $129 \pm 14\%$ and $144 \pm 3\%$, respectively. They were then given i.t. injections of dynorphin-(1-13) 5 nmol every 2 h for 5 times. The average analgesic responses following the successive injections were
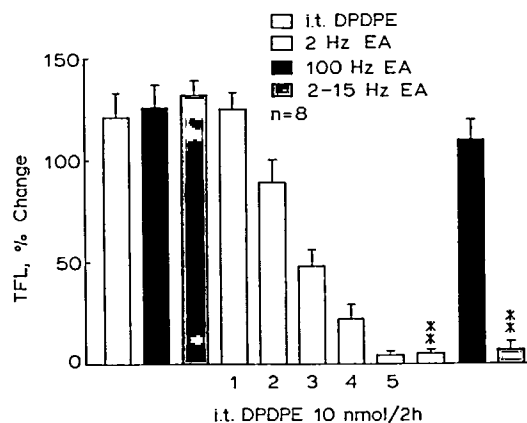

Fig. 2. Tolerance to DPDPE-produced analgesia and cross-tolerance to analgesia induced by EA of different frequencies. The protocol is the same as in Fig. 1, except that DPDPE 10 nmol (light grey columns) was used instead of ohmefentanyl. Annotation same as in Fig. 1.
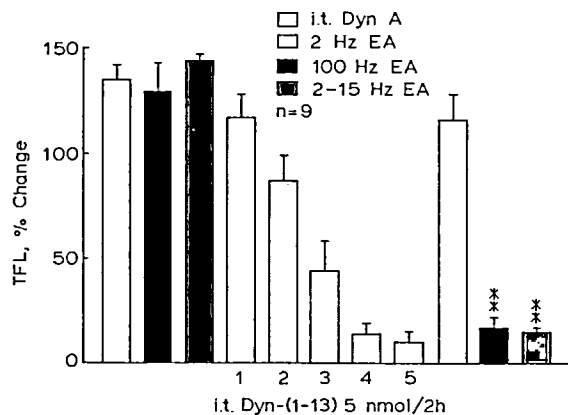
Fig. 3. Tolerance to dynorphin-(1-13)-produced analgesia and cross-tolerance to analgesia induced by EA of different frequencies. The protocol is the same as in Fig. 1, except that dynorphin-(1-13) (light grey columns) was used instead of ohmefentanyl. Annotation same as Fig. 1.
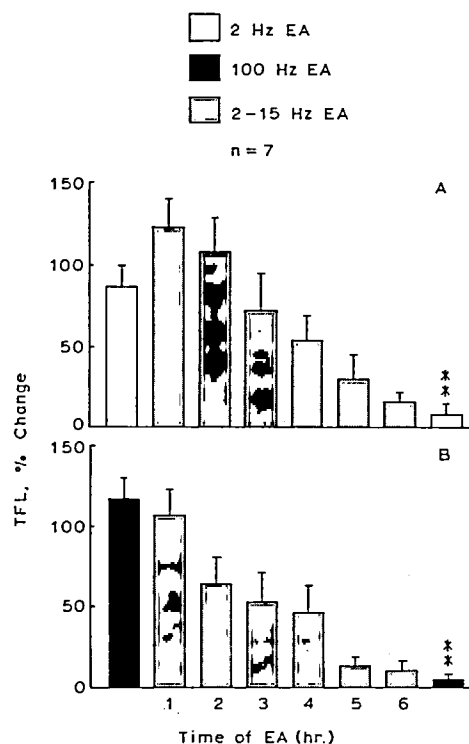

Fig. 4. Tolerance to 2–15 Hz-EA (dark grey columns) analgesia and cross-tolerance to either 2 Hz-EA (white columns) or 100 Hz-EA (black columns) analgesia. Panel A: rats (n = 7) were given 2 Hz-EA for 30 min, and the analgesic effect was measured by the percentage change of TFL. They were then given 2–15 Hz-EA for 6 h, with the TFL measured at the end of each hour. The frequency of EA was then shifted to 2 Hz, and the TFL measured at the end of a 30 min stimulation period. Similar experiments were performed using 100 Hz-EA instead of 2 Hz-EA and the results are shown in panel B. The frequency of EA is indicated in the corresponding boxes. **$P < 0.01$ (paired t-test) as compared to the corresponding value taken prior to the development of 2–15 Hz-EA tolerance.

117 ± 11%, 87 ± 12%, 44 ± 14%, 14 ± 5% and 10 ± 5%, respectively, showing the development of tolerance to dynorphin-(1-13). At this point, rats were given EA stimulation of 2 Hz, 100 Hz and 2–15 Hz, respectively. Rats made tolerant to the κ-agonist dynorphin-(1-13) were still reactive to 2 Hz-EA (116 ± 12%, $P > 0.05$ as compared to 135 ± 7% by the paired t-test), suggesting that no significant cross-tolerance was found between 2 Hz-EA analgesia and dynorphin-produced analgesia. In the mean time there was a diminished response to 100 Hz- and 2–15 Hz-EA (17 ± 5% and 15 ± 2%, $P < 0.01$ as compared to 129 ± 14% and 144 ± 3%, respectively), suggesting that rats made tolerant to dynorphin-(1-13) showed cross-tolerance to either 100 Hz- or 2–15 Hz-EA.

Tolerance to 2–15 Hz-EA analgesia and cross-tolerance to either 2 Hz- or 100 Hz-EA analgesia

2–15 Hz-EA stimulation was given to a group of 7 rats continuously for 6 h and TFL measured at the end of each hour. The results are shown in Fig. 4A. The analgesic effect decreased gradually from 123 ± 17% to 16 ± 6% at the end of the 6th hour, indicating the development of EA tolerance. At this point, the effect of 2 Hz-EA stimulation was tested and found to be only 8 ± 7%, which was significantly lower than the effect of 2 Hz-EA analgesia (87 ± 13%, paired t-test, $P < 0.01$) taken before the tolerance to 2–15 Hz-EA. Thus, a complete cross-tolerance existed between 2–15 Hz- and 2 Hz-EA analgesia.

Another group of 7 rats were given 100 Hz-EA stimulation for 30 min, the percentage change of TFL was 117 ± 13%. The rats were then given 2–15 Hz-EA continuously for 6 h. The analgesic effect decreased

gradually from 107 ± 16% to 10 ± 6% at the end of 6 h, suggesting the development of EA tolerance as shown in Fig. 4B. The frequency of EA was then shifted to 100 Hz, and the TFL was measured at the end of the 30-min stimulation period. The analgesic effect was only 5 ± 3% (paired t-test, $P > 0.05$ as compared to 10 ± 6%; $P < 0.01$ as compared to 117 ± 13%). The results suggested that rats made tolerant to 2–15 Hz-EA analgesia showed complete cross-tolerance to 100 Hz-EA analgesia.

Tolerance to either 2 Hz- or 100 Hz-EA analgesia and cross-tolerance to 2–15 Hz-EA analgesia

Eighteen rats were evenly divided into two groups. The first group was given 2–15 Hz-EA for 30 min, which produced an increase of TFL of 134 ± 7%. They were then given 2 Hz-EA continuously for 6 h. The analgesic effect determined hourly was 135 ± 8%

at the first sessions and $9 \pm 3\%$ at the last session. At this point, the frequency of EA stimulation was shifted to 2–15 Hz. At the end of 30-min stimulation period, the percentage change of TFL reached $84 \pm 13\%$ (paired t-test, $P < 0.01$ as compared to $9 \pm 3\%$ and $P < 0.05$ as compared to $134 \pm 7\%$). The results suggest that 2–15 Hz-EA analgesia had decreased but not completely disappeared in rats made tolerant to 2 Hz-EA analgesia (Fig. 5A).

A similar experiment was performed using 100 Hz-EA instead of 2 Hz-EA. The results are shown in Fig. 5B. In rats rendered tolerant to 100 Hz-EA analgesia, the analgesic effect of 2–15 Hz-EA was found to be $87 \pm 19\%$, which was significantly lower than the effect of 2–15 Hz-EA analgesia ($148 \pm 2\%$, $P < 0.05$) taken prior to 100 Hz-EA tolerance. In the meantime it was significantly higher than the analgesic effect of 100 Hz-EA ($10 \pm 4\%$, $P < 0.01$) at its sixth session, indicating that rats made tolerant to 100 Hz-EA analgesia were still partially effective to 2–15 Hz-EA analgesia.
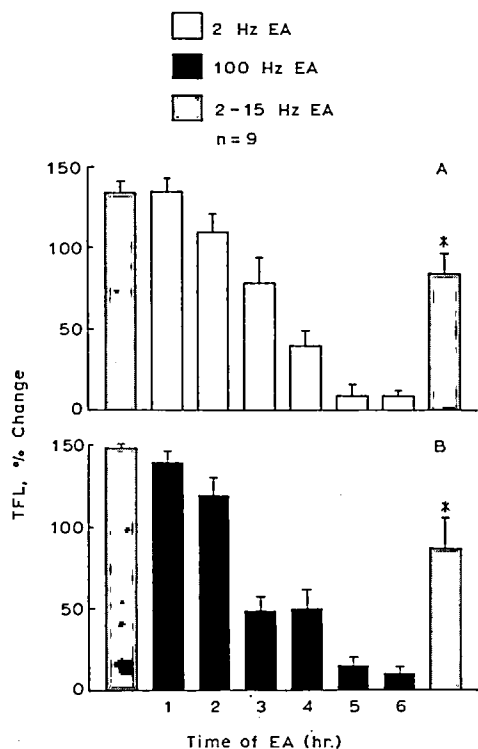
Results mentioned above indicated that rats rendered

Fig. 5. Tolerance to either 2 Hz-EA (white columns) or 100 Hz-EA (black columns) analgesia and cross-tolerance to 2–15 Hz-EA (dark grey columns) analgesia. The protocol was the same as in Fig. 4, except that 2 Hz- or 100 Hz-EA was given continuously for 6 h to induce tolerance, and its effect on 2–15 Hz-EA analgesia was checked thereafter. *$P < 0.05$ as compared to the corresponding value taken prior to the development of 2 Hz- or 100 Hz-EA tolerance by a paired t-test.

tolerant to either 2 Hz- or 100 Hz-EA analgesia were still reactive to 2–15 Hz-EA, although the efficacy was cut down by about 40%.

Another group of 10 rats were given 2–15 Hz-EA stimulation for 30 min, which produced an increase in TFL of $103 \pm 13\%$. They were left in the holders, given no stimuli, and TFL was measured hourly for 6 times. The percentage changes of TFL fluctuated within the range of $-4 \pm 2\%$ to $3 \pm 2\%$, showing no significant changes with time as analyzed by the t-test. The rats were then given 2–15 Hz-EA for 30 min, which produced an increase of TFL of $109 \pm 14\%$ ($P > 0.05$ as compared to $103 \pm 13\%$), showing that after 7 h of staying in the holder the rats were still reactive to 2–15 Hz-EA stimulation.

DISCUSSION

In the present study, the cross-tolerance technique was used to analyze whether the endogenously released opioid ligands are acting on the same type of receptor as the ones activated by exogenous ligands. Thus, DPDPE has been generally accepted as the specific δ-agonist[17] and dynorphin A the specific κ-agonist[22]. For the specific μ-agonist there are several candidates, including DAGO[5], PL017[2,20] and ohmefentanyl[23,25]. The latter is a newly developed non-peptide μ-specific opioid agonist, its IC_{50} for competing against [^{3}H]DAGO binding was only 0.19 nmol/l whereas that for [^{3}H]DPDPE was 89.1 nmol/l, with a ratio of 481 preferring μ to δ in rat brain membrane[23,25]. After a thorough comparison, Goldstein and Naidu concluded that OMF could be superior to DAGO as a μ-selective ligand[6]. For these reasons we have chosen to use OMF instead of DAGO as μ-selective agonist in the present study.

Repeated intrathecal injection of OMF (15 pmol), DPDPE (10 nmol) or dynorphin-(1-13) (5 nmol) in rats resulted in the development of selective tolerance to the μ-, δ-, or κ-receptors, respectively. These rats were tested for their reactivity to EA of different frequencies. Since in the Latin square test the order of 2 Hz-, 100 Hz- and 2–15 Hz-EA has no significant influence upon their effects of EA analgesia (unpublished data), we have used the order: 2 Hz, 100 Hz and 2–15 Hz. The results are summarized in Table I. It is clearly obvious that μ- and δ-receptors seem to work together for 2 Hz-EA analgesia in contrast to the κ-receptor which mediates the 100 Hz-EA effect. This is in line with the previous findings[3,19] that 2 Hz-EA increased the release of spinal met-enkephalin which showed considerable affinity both to μ- and δ-opioid recep-

TABLE I

Cross-tolerance between opioid analgesia and EA analgesia

Primary tolerance to opioid analgesia	Cross-tolerance to EA analgesia		
	2 Hz	100 Hz	2–15 Hz
μ	+	−	+
δ	+	−	+
κ	−	+	+

+, cross-tolerance; −, no cross-tolerance.

tors[13,15,16], whereas 100 Hz-EA increased the release of spinal dynorphin which is the specific agonist for κ-receptor[22]. The interesting finding is that 2 Hz-EA analgesia stops working when either the μ- or δ-receptor is blocked, suggesting that a simultaneous activation of μ- and δ-receptor is mandatory for full functioning of the effect of 2 Hz-EA. Similarly, the full functioning of 2–15 Hz-EA (D-D mode) depends on the simultaneous activation of the μ-, δ- and κ-receptors. Masking the normal functioning of any one of the 3 varieties of opioid receptors leads to the total abolishment of the 2–15 Hz-EA analgesia. A synergistic effect has been shown between any two of the three opioid agonists: morphine, met-enkephalin and dynorphin-(1-13)[11,14]. An even stronger synergism among μ-, δ- and κ-agonists has been proposed, but not yet proved. The data shown above suggest that 2 Hz-EA analgesia is mediated by μ- and δ-receptors, 100 Hz-EA analgesia by the κ-receptor, and 2–15 Hz-EA analgesia by the combined activation of μ-, δ- and κ-receptors. A summary is shown in Table I.

The above hypothesis could well account for the observations that rats made tolerant to 2–15 Hz-EA showed cross-tolerance to either 2 Hz- or 100 Hz-EA, and vice versa. It was interesting to note that rats made tolerant to μ-agonist OMF, δ-agonist DPDPE or κ-agonist Dynorphin (1-13) (Q2h × 5) lost their response to 2–15 Hz almost completely (>90%); whereas rats rendered tolerant to 2 Hz- or 100 Hz-EA showed only 40% reduction in their response to 2–15 Hz-EA. This could be accounted for by the difference in the degree of tolerance. It is conceivable that the tolerance induced by repeated intrathecal injection (Q2h × 5) of opioid agonist should be much more severe than that induced by physiologically released opioid peptides as triggered by 2 Hz- or 100 Hz-EA stimulation. While the mechanisms underlying this phenomenon may still be under debate, its clinical implication is straight forward. We have shown previously that rats made tolerant to 2 Hz-EA were still reactive to 100 Hz-EA and vice verse[3,19]. If 2 Hz- (or 100 Hz-) EA is used and the patient finally develops tolerance to it, one could change the frequency to 100 Hz (or 2 Hz), or change the mode of stimulation from 'continuous' to 'dense-and-disperse' (D-D) and regain analgesic effect. However, if 2–15 Hz-EA was chosen at first, and the patient becomes tolerant to it, then neither 2 Hz nor 100 Hz would be effective.

Another issue worth mentioning is that there has been controversy concerning the validity of the antinociceptive effect of dynorphin first reported by Han and colleagues[9]. Since a large dose of dynorphin (i.t., >10 nmol) induces hind limb paralysis in rats, it was claimed that the increase in tail-flick latency following i.t. injection might have resulted from motor neuron intoxication and paralysis[1,10]. If this were true, the increase in tail-flick latency induced by i.t. injection of dynorphin-(1-13) (5 nmol) would have led to motor neuron necrosis and complete motor paralysis upon repeated administration, which was not the case. We observed in the present study that rats injected with dynorphin-(1-13) (5 nmol) had normal motor function at 10, 20, 30 min and 2 h post-injection. Histologic examination revealed no dead neurons either in dynorphin-(1-3)-treated rats or in saline-treated rats. Results shown in Fig. 3 indicate that instead of inducing motor paralysis which would have resulted in an indefinite prolongation of TFL, the effect of dynorphin-(1-13) waned on repeated injections, suggesting the development of tolerance to dynorphin-induced analgesia. A cross-tolerance of dynorphin-(1-13) analgesia toward 100 Hz- but not 2 Hz-EA analgesia further argues for a κ-specific analgesic effect induced by exogenously administered (small dose) and endogenously released dynorphin.

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