INFLUENCE OF TOLERANCE TO AND PHYSICAL DEPENDENCE ON OPIOID ANALGESICS ON CORTICAL EVOKED POTENTIAL IN RATS

Akira SHIMADA and Tomoji YANAGITA
Department of Pharmacology, Preclinical Research Laboratories, Central Institute for Experimental Animals, 1433 Nogawa, Miyamae-ku, Kawasaki 213, Japan

Accepted June 12, 1982

Abstract—The influence of single and repeated doses of morphine, pentazocine, and chlorpromazine on the latent time of cortical evoked potentials was observed in rats. The evoked potentials were induced by electrical stimulation of the sciatic nerve under the condition of curarization and artificial respiration. The evoked potential consisted of 4 phases. The latent time of the N2 component was prolonged by single dose administration of morphine at 5 mg/kg, s.c., pentazocine at 15 mg/kg, s.c., or chlorpromazine at 2 mg/kg, s.c. The effects of morphine and pentazocine were antagonized by naloxone but that of chlorpromazine was not. By repeated administration of morphine at 5 mg/kg or pentazocine at 15 mg/kg twice daily for 4 weeks, the prolongation of latent time tended to disappear gradually. Further, in these rats the latent time became shorter than that of normal rats after naloxone administration or natural withdrawal for 18 hr. Thus the development of tolerance to and physical dependence on these drugs were demonstrated by recovering and shortening of the latent time, even when withdrawal signs were not apparently observed in the rats' gross behavior. On the other hand, repeated administration of chlorpromazine at 2 mg/kg twice daily for 4 weeks did not produce such shortening of the latent time as was observed with morphine and pentazocine. These results indicate that the evoked potential can serve as a sensitive parameter for observation of the development of tolerance to and physical dependence on opioid analgesics in rats.

It is well known that repeated administration of opioid analgesics leads to the development of tolerance to and physical dependence on these drugs in the mouse (1), rat (2-4), dog (5, 6), monkey (7-9), and man (10, 11). As indices for observing physical dependence on opioid analgesics in the main, body temperature and some behavioral signs have been used in experimental animals. In an early observation using rats, Kaymakcalan and Woods (12) reported the withdrawal signs in rats to be sedation, loss of both muscular rigidity and exophthalmos, and marked increase in intestinal activity as evidenced by increased defecation, soft stools and occasional diarrhea. Bläsig et al. (13) examined and analyzed the frequency of occurrence of various quantifiable signs such as exploring, jumping, flying, wet-dog shaking, teeth chattering, and writhing in rats in relation to varying degrees of dependence. Regardless of this, change in
Body weight has been regarded by many investigators as the most objective and reliable parameter for observation of physical dependence on opioid analgesics in rats. Unfortunately, however, body weight change is not a very sensitive parameter, particularly when physical dependence is developed on some of the synthetic opioid analgesics such as pethidine and pentazocine. Therefore, the use of evoked potential as an objective and sensitive parameter for observing physical dependence on analgesics in rats was investigated in the present study.

MATERIALS AND METHODS

Thirty Sprague-Dawley rats (Nihon Clea Co., Japan) that had been housed in quarters regulated with respect to temperature, humidity and lighting cycle for 1 week or more were used in this experiment. The rats were fed a solid diet (CA-1, Nihon Clea Co., Japan) with tap water ad lib. The animals were randomly divided into 6 groups of 5 rats each.

The observation of evoked potentials was carried out as follows. Each rat was first immobilized by d-tubocurarine chloride (Amelizol®, Yoshitomi Pharmaceutical Co., Japan) at a dose of 0.1 mg/kg, i.v. Immediately thereafter, a polyethylene cannula was inserted to the trachea for artificial respiration. Artificial respiration was maintained at a tidal volume of 1 ml/breath and a breath rate of 90/min. A small animal respirator (Natsume Co., Japan) was used for this purpose. The rat’s head was held immobile by a stereotaxic instrument (Takahashi Co., Japan), and a cashew resin-coated monopolar silver ball electrode was placed through a hole in the skull over the dura mater while a reference electrode was placed in the frontal sinus. The sciatic nerve, cut off at the lower end of the thigh region, was electrically stimulated by 20 single square pulses, 0.1 msec in duration and 2 sec apart. The potentials evoked in the contralateral cerebral cortex were amplified by an electroencephalograph recorder (ME-950, Nihon Kohden Co., Japan) and cumulated by an Addscope® (ATAC-250, Nihon Kohden Co., Japan) and an averaged noise-analyzed response obtained every 20 electrical stimuli was registered on an XY recorder (Type 3077, Yokokawa Denki Co., Japan).

During the observation period, the electrocardiograph was simultaneously monitored by a polygraph (RM-150, Nihon Kohden Co., Japan), and added doses of 0.3 mg/kg of d-tubocurarine were injected i.m. to the left hindlimb of the rat at 30-min intervals. Drugs were administered with an injection volume of 2 ml/kg for pentazocine and 1 ml/kg for the others. The drugs were prepared at least every 7 days.

1. Observation in normal untreated rats: Rats weighing between 170 and 340 g were used. First, the evoked potentials were recorded prior to the administration of drugs as the normal state. After stable responding was obtained, the rats were subcutaneously administered single doses of 5 mg/kg of morphine hydrochloride (Takeda Pharmaceutical Co., Japan), 15 mg/kg of pentazocine lactate (Winthrop Laboratories, Japan), or 2 mg/kg of chlorpromazine hydrochloride (Yoshitomi Pharmaceutical Co., Japan), and the influence on the evoked potential was observed for 60 min. Naloxone hydrochloride (Sankyo Central Research Laboratories, Japan) was then administered at 2 mg/kg, s.c., to the same rats at 60 min after administration of the drugs, and the influence of naloxone on the drug effects was observed.

2. Observation in repeatedly treated rats: Rats weighing between 122 and 247 g were used. The rats subcutaneously received doses of 5 mg/kg of morphine, 15 mg/kg of pentazocine, and 2 mg/kg of chlorpromazine twice daily for 4 weeks. On holidays, only the
morning dose was given at twice the normal dose of the drugs. Rats treated with these drugs for 4 weeks were abruptly withdrawn for 18 hr after the last dose. At this point, the effect of readministration of the usual test dose of the drugs on the evoked potential as well as the antagonism of 2 mg/kg of naloxone were observed in the same manner as that described in section 1. During the repeated administration period, body weight and behavioral signs were observed every day. These were also observed at 18 hr after the last dose of the drugs.

RESULTS

1. Morphine: After single-dose administration of morphine at 5 mg/kg, the rats showed decrease in spontaneous motor activity and suppression of the touch response, but these were not very clear. The evoked potentials obtained from the contralateral cortex consisted of 2 waves with 4 phases, including positive and negative components, in normal untreated rats. They were labeled \( P_1 \), \( N_1 \), \( P_2 \) and \( N_2 \) (Fig. 1), and the ranges of the latent times of these phases from electrical stimulation of the sciatic nerve until the peak of these phases were 25.0 to 37.5, 45.0 to 65.0, 115.0 to 150.0, and 180.0 to 225.0 msec, respectively. While their amplitudes were subject to wide fluctuation, both the latent times and amplitudes of the \( P_1 \) and \( N_1 \) components were hardly influenced by single doses of morphine. On the other hand, a decrease in the amplitude of \( P_2 \) and prolongation of the latent times of both \( P_2 \) and \( N_2 \), especially the \( N_2 \) component, were observed. The average latent time of the \( N_2 \) component before morphine administration was 198.0±8.5 (mean±S.E.) msec. It was prolonged from 15 min, increasing to 260±9.4 msec at 60 min after the administration of morphine. With the administration of 2 mg/kg naloxone after observation of the effects of morphine for 60 min, these effects were antagonized between 30 and 60 min after naloxone administration (Table 1). Since the prolongation of the latent time of the \( N_2 \) component produced by a single dose of morphine was observed as a clear-cut effect on the evoked potential, the influence of repeated administration of drugs on the latent time was examined regarding the \( N_2 \) component. In rats repeatedly treated with morphine, decrease of spontaneous motor activity was shown before each administration from about 2 weeks after the initial dose, especially before the morning dose. However, spontaneous motor activity was markedly increased immediately before and after administration of morphine to rats which had been repeatedly treated and showed diminution of the activity, something that was not observed in rats administered the initial dose. Furthermore, these rats became hypersensitive to the irritative stimulation induced by the needle when the drug was administered s.c. The body weight of the rats increased during the period of repeated administration of morphine for 4 weeks. The average body weight when the last dose was administered was 334.2±5.2 g (mean±S.E.), while 18 hr after the last dose, it was 337.4±3.6 g. Loss of body weight resulting from the withdrawal of morphine was not observed in the groups of

![Fig. 1. Cortical evoked potential in a normal rat.](image)
### Table 1. Effect of morphine on the latent times of the evoked potentials and antagonism by naloxone in normal untreated rats

<table>
<thead>
<tr>
<th>Components</th>
<th>Before Morphine</th>
<th>Naloxone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>P1</td>
<td>28.5</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>± 2.9</td>
<td>2.8</td>
</tr>
<tr>
<td>N1</td>
<td>58.0</td>
<td>59.4</td>
</tr>
<tr>
<td></td>
<td>± 3.4</td>
<td>± 2.5</td>
</tr>
<tr>
<td>P2</td>
<td>130.0</td>
<td>130.0</td>
</tr>
<tr>
<td></td>
<td>± 6.1</td>
<td>± 10.8</td>
</tr>
<tr>
<td>N2</td>
<td>198.0</td>
<td>223.0</td>
</tr>
<tr>
<td></td>
<td>± 8.5</td>
<td>± 7.8</td>
</tr>
</tbody>
</table>

Each value shows the mean and standard error. The dose of 5 mg/kg of morphine was administered s.c. at 0 min while naloxone was administered s.c. after 60 min.

### Table 2. Influence of discontinuation of drugs on body weight in repeatedly treated rats

<table>
<thead>
<tr>
<th>Drugs</th>
<th>1st</th>
<th>7th</th>
<th>14th</th>
<th>21st</th>
<th>28th</th>
<th>18 hr after last dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>229.2±6.1</td>
<td>247.6±9.3</td>
<td>286.4±9.6</td>
<td>312.0±6.8</td>
<td>334.2±5.2</td>
<td>337.4±3.6</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>166.0±12.8</td>
<td>205.4±12.0</td>
<td>249.2±10.2</td>
<td>288.4±9.2</td>
<td>323.2±8.5</td>
<td>327.6±8.5</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>164.4±16.0</td>
<td>191.6±11.3</td>
<td>219.2±12.4</td>
<td>260.0±13.8</td>
<td>290.0±12.1</td>
<td>293.2±12.9</td>
</tr>
</tbody>
</table>

Each value shows the mean and standard error.
Table 3. Influence of repeated administration of morphine on the $N_2$ latent times of the evoked potentials in rats

<table>
<thead>
<tr>
<th>No of animals</th>
<th>Before</th>
<th>Morphine (0 15)</th>
<th>Latent time of $N_2$ component (msec)</th>
<th>Naloxone</th>
<th>Time after morphine administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>40 60 75 90 120 150 180 240 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>110.0</td>
<td>260.0</td>
<td>270.0 270.0 225.0 115.0 110.0 210.0 245.0 250.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>140.0</td>
<td>155.0</td>
<td>155.0 195.0 150.0 115.0 140.0 110.0 180.0 170.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>105.0</td>
<td>220.0</td>
<td>212.5 225.0 80.0 100.0 110.0 95.0 92.5 240.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>190.0</td>
<td>162.0</td>
<td>197.5 200.0 145.0 115.0 130.0 122.5 127.5 137.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>105.0</td>
<td>235.0</td>
<td>247.5 245.0 145.0 115.0 115.0 105.0 140.0 120.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>144.0</td>
<td>206.5</td>
<td>216.5 227.0 149.0 112.0 121.0 128.5 157.0 180.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.E.</td>
<td>17.0</td>
<td>20.6</td>
<td>20.0 14.0 23.0 3.0 6.0 20.9 26.1 20.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rats were tested 18 hr after the last dose of morphine. Values represent the latent times of individual rats with the mean and standard error. The test dose of 5 mg/kg of morphine was administered s.c. at 0 min while 2 mg/kg of naloxone was administered s.c. after 60 min.
rats treated with morphine for 4 weeks (Table 2). Similarly, typical behavioral withdrawal signs and symptoms such as wet-dog shaking, teeth-chattering, jumping and/or convulsions were not observed during the experimental period. Table 3 shows the changes in the latent times in individual animals which had repeatedly received morphine for 4 weeks and were withdrawn for 18 hr, as well as the mean values for their latent times. The comparison of the average latent times between normal untreated and repeatedly morphine-treated rats is shown in Fig. 2. The average latent time 18 hr after the last dose of morphine was 144.0±17.0 msec. It was shorter than that observed before morphine administration in normal untreated rats. Application of the t-test yielded a P value of <0.05 for the changes in latent time before morphine administration in the normal untreated group compared with the 4-week treated and withdrawn group. There was considerable variation in the latent times of individual animals. When a single dose of 5 mg/kg of morphine was readministered to the withdrawn rats at 18 hr after the last dose, the latent time was prolonged beginning 15 min after the administration. It was prolonged to 227.0±14.0 msec by 60 min after the administration, but was still shorter than that observed after morphine administration in normal untreated rats. The latent time that had been prolonged by the administration of morphine was markedly shortened 30 min after the administration of naloxone, the average latent time then being 112.0±3.0 msec. This was even shorter than that observed before morphine administration in normal untreated rats (P<0.01).

2. Pentazocine: In normal untreated rats, a single dose of 15 mg/kg of pentazocine showed no effects in the observation of gross behavior. The average latent time of the N2 component before drug administration was 190.0±12.2 msec. By the administration of pentazocine, it was prolonged to 253.0±18.3 msec at 60 min after the administration. This effect caused by the pentazocine was antagonized by naloxone.

In rats repeatedly treated with pentazocine, increased spontaneous motor activity was observed after pentazocine administration between the 3rd and 5th days of treatment. By 1 to 2 weeks after the initial dose, one rat administered pentazocine showed hyperactivity, jumping, slight tail raising, enlargement of the pulpebral opening, exophthalmos, increasing startle response, fighting, and hypersensitivity to other environmental stimuli. By the 7th day of treatment, transient convulsions were elicited by sudden loud noises, and abnormal gait was observed in one out of 5 rats about 15 min after the morning dose. By 3 to 4 weeks after the initial dose, the rats showed a decrease in spontaneous motor activity and ptosis before the morning and evening doses, especially.
Fig. 3. Influence of repeated administration of pentazocine on the latent time of the N₂ component of the evoked potential in rats. Values represent the mean and standard error. The test dose of 15 mg/kg of pentazocine was administered s.c. at 0 min, while 2 mg/kg of naloxone was administered s.c. after 60 min. ---; normal untreated rats; ---O--; repeatedly treated rats. The latent time after administration of pentazocine was shorter than that in normal untreated rats. The latent time 18 hr after withdrawal as well as after naloxone administration was also shorter than that observed in normal untreated rats.

before the morning dose. The average body weight when the last dose was administered was 323.2±8.5 g, and that at 18 hr after the last dose was 327.6±8.5 g. Loss of body weight resulting from the withdrawal of pentazocine was not observed (Table 2). The average latent time 18 hr after the last dose of pentazocine was 171.0±18.8 msec, which was short in comparison with that observed before the administration of pentazocine in normal untreated rats. By readministration of 15 mg/kg of pentazocine, it lengthened to 218.0±28.2 msec by 60 min after administration, but was still shorter than that noted after administration of pentazocine in normal untreated rats. The latent time that had been prolonged by readministration of pentazocine was shortened by the administration of naloxone, becoming shorter than that observed before administration of pentazocine in normal untreated rats (Fig. 3).

3. Chlorpromazine: By a single dose of 2 mg/kg of chlorpromazine, rats frequently showed grooming and hyperactivity 3 to 5 min after administration of the drug. Thereafter, ptosis and decrease in spontaneous motor activity were observed, followed by precipitation of the cataleptic effect in all rats about 15 min later. These effects caused by chlorpromazine lasted 3 to 4 hr or more. However, the occurrence of the startle response elicited by handling or sudden loud noises was not disturbed during this period. The average latent time of the N₂ component before administration of the drug was 206.0±23.9 msec. It lengthened to 230.0±13.0 msec at 60 min after administration of chlorpromazine. This effect was not antagonized by naloxone.

In rats repeatedly treated with chlorpromazine for 4 weeks, it was found that the behavioral effects as observed in rats administered the initial dose of chlorpromazine were hardly influenced by repetitive doses of the drug. A few rats showed soft stools and hyperirritability 18 hr after the last dose. The average body weight when the last dose was administered was 290.0±12.1 g, and that in rats withdrawn for 18 hr was 293.2±12.9 g. The body weight in rats withdrawn for 18 hr did not decrease with the discontinuation of chlorpromazine (Table 2). The average latent time 18 hr after abrupt withdrawal of chlorpromazine was 207.0±15.7 msec. It was approximately equal to that observed before administration of chlorpromazine in normal untreated rats. It was prolonged to 244.0±17.7 msec at 60 min after readministration of 2 mg/kg of chlorpromazine, a latent time that was also approximately equal to that observed after administration of chlorpromazine in normal untreated rats. The latent time prolonged by the readministration of chlorpromazine was not antagonized by
DISCUSSION

The evoked potential studied here consisted of 2 waves with 4 phases induced by electric stimulation of the sciatic nerve of the rat. Both the P2 and N2 components of the evoked potential were prolonged by single doses of morphine, pentazocine, and chlorpromazine. It has been reported that the evoked potential induced by electric stimulation of the sciatic nerve in cats is suppressed by exposure to morphine and that morphine has the property of suppressing the diffuse thalamic projection system, the ascending reticular activating system, and the thalamo-cortical reflection system (14). Furthermore, Yasuhara (15) and Shigenaga (16) have shown in rats and cats that the evoked potential (long latent time) induced by electric stimulation of the sciatic nerve and/or tooth pulp was supressed by morphine and that the short latent time was not sensitive to morphine since it resulted from the specific sensory system. Pentazocine also had a prolonging effect on the evoked potential. Among investigations concerning the site of action of pentazocine on the brain, Yanagida and Yamamura (17) have reported that the inhibition of the non-specific thalamic nucleus was induced by directly injecting pentazocine into the brains of cats. From these findings, it may be supposed that the actions of morphine and pentazocine as changes in the P2 and N2 components are mainly due to the suppressing effects of the drugs on the non-specific sensory system, but not due to suppression of the specific sensory system. However, the mechanism of action of chlorpromazine is not evident in evoked potential.

Rats repeatedly treated with morphine for 4 weeks showed slight diminution of spontaneous motor activity before both morning and evening doses from about 2 weeks after the initial dose of morphine. This might be seen as an apparent withdrawal sign as described by Kaymakcalan and Woods (12). Various theories have put forth to explain the mechanisms of tolerance and physical dependence such as the dual action theory (18), the denervation theory (19), and the redundancy theory (20, 21). Common among these theories is the view that tolerance develops only to the central nervous system depressant effects, and development of withdrawal signs is due to a state of latent hyperexcitability in the central nervous system. Recently, McClung et al. (22) have reported that tolerance was induced to morphine's increasing effect on amplitude in observations of the sensory-evoked potentials induced by acoustic stimuli in rats. This finding has indicated that tolerance can be observed by using a technique for the observation of the evoked potential of selected regions of the central nervous system, but whether this was the case with physical dependence was not
studied in the experiment. The phenomenon that the prolonged latent time after administration of morphine in rats repeatedly treated with morphine was shorter than that observed in normal untreated rats is taken as indicating that tolerance to the effect of morphine was developed by repetitive doses of the drug. When morphine was abruptly withdrawn in repeatedly treated rats, the latent time was even shorter than that observed before administration of morphine in normal untreated rats. This may be taken as the reason for the appearance of withdrawal signs. It is thought that a mechanism similar to functional hypertrophy of the synapse as described in the redundancy theory (20) may be involved in the development of morphine tolerance and physical dependence.

Pentazocine also had a prolonging effect on the latent time of the N2 component of the evoked potential similar to that of morphine. The decreased spontaneous motor activity and/or ptosis observed before administration of pentazocine in the fourth week of repeated treatment with the drug may be an apparent, though negligible, pentazocine withdrawal sign. The latent time prolonged by readministration of pentazocine in withdrawn rats was shorter than that observed after administration of pentazocine in normal untreated rats, thus indicating that tolerance to pentazocine has been developed. The latent times 18 hr after the last dose as well as after the administration of naloxone were shorter than that observed before administration of pentazocine in normal untreated rats. This indicates the appearance of the withdrawal sign as in morphine withdrawn rats. Yanagita et al. (23, 24) have reported that monkeys or rats repeatedly treated with pentazocine showed much weaker withdrawal signs than those of morphine. This finding indicates that the physical dependence-producing potential of pentazocine is less than that of morphine. In the present study, it was found that the withdrawal signs of drugs with relatively low physical dependence potential such as pentazocine may be measured using the method herein described, since the shortening of the latent time was observable even when apparent behavioral withdrawal signs were not observed.

It has been generally thought that chlorpromazine is a central nervous system depressant that produces tolerance to its sedative effects (25). Matsuki and Iwamoto (26) have reported that in the rat, tolerance to chlorpromazine develops with respect to the inhibitory action on the conditioned avoidance response. Concerning the development of physical dependence, however, the opinions are many and varied among the investigators. Boyd (27) showed that animals developed tolerance to the lethal and central nervous system depressant effects of the drug and showed hyperkinesia, diarrhea, and death as withdrawal signs. Deneau et al. (28), in an experiment on intravenous self-administration of chlorpromazine in monkeys, reported that the drug is not likely to produce physical dependence. Yanagita and Takahashi (29) have further tested the dependence liability of 10 sedative-hypnotic agents in monkeys and classified chlorpromazine among the agents that do not have a physical dependence-producing liability. The rats treated for 4 weeks with chlorpromazine showed little or no tolerance to behavioral signs nor any striking behavioral withdrawal signs. However, a few rats showed soft stools and hyperirritability 18 hr after the withdrawal of chlorpromazine. Whether or not these signs were attributable to the withdrawal of the drug is not known, but as far as the evoked potential is concerned, no shortening of the latent time was found in the withdrawal period. Therefore, the observation of the latent time of evoked potential appears to be a good parameter to differentiate the withdrawal manifestation
due to physical dependence from that not due to physical dependence. This effect by chlorpromazine was not altered by repeated administration of the drug. Furthermore, the latent time at 18 hr after the last dose and after naloxone was just as long as that of the normal untreated rats. These findings indicate that tolerance was not developed to the effect on the evoked potential and, as previously reported by Deneau et al. (28) and by Yanagita and Takahashi (29), chlorpromazine may not have any physical dependence-producing liability.

The latent time of the evoked potential was markedly shortened in morphine- and/or pentazocine-withdrawn rats, but not in chlorpromazine-withdrawn rats. Therefore, it can be said that the shortening of the latent time of the evoked potential was observed only in withdrawal of the drugs with physical dependence potential and that observation of the latent time of the cortical-evoked potentials induced by electric stimulation of the sciatic nerve in rats can be a sensitive parameter for observing the withdrawal signs of opioid analgesics. It can also be a useful method for elucidating the mechanism of the development of tolerance to and physical dependence on opioid analgesics.

REFERENCES


