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Proconvulsant effects of tramadol and morphine on pentylenetetrazol-induced seizures in adult rats using different routes of administration

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ABSTRACT

Tramadol is frequently used as a pain reliever. However, it has been sometimes noted to have the potential to cause seizures. Because of its dual mechanism of action (both opioid and nonopioid), the adverse effect profile of tramadol can be different in comparison with single-mechanism opioid analgesics, such as morphine. In the present study, the facilitatory effects of tramadol and morphine on pentylenetetrazol-induced seizures using different routes of administration were compared in rats. Adult female rats were divided into six groups and continuously received saline, morphine, or tramadol on a daily basis for 15 days [gavage (PO) or intraperitoneal (IP)]. An increasing dose of morphine and tramadol was used to prevent resistance to repetitive dose (20–125 mg/kg). Following one week of withdrawal period and 30 min before the seizure induction (PTZ = 80 mg/kg, IP), each group of rats was further divided into subgroups that received saline, morphine, or tramadol for the second time on the 22nd day of the experiment. Results showed that, while morphine, tramadol, and their administration had different effects on seizure behaviors, both acute and chronic administrations of morphine and tramadol potentiated PTZ-induced seizures. However, there was no significant difference between morphine and tramadol in terms of seizure severity. Effects of morphine and tramadol on PTZ-induced seizures were also stable following one week of withdrawal. In conclusion, this study indicated similar severity in the proconvulsant effect of morphine and tramadol on PTZ-induced seizures, which might depend on their similar effects on GABAergic pathways.

1. Introduction

Epilepsy is characterized by recurrent, unprovoked seizures with any immediately identifiable cause [1–3]. It is a common neurological disorder that affects individuals of all ages [2]. In recent years, some animal models of epilepsy have been developed, which include genetic animal models, chemical-induced epilepsy models, and kindling models [4,5]. These models have played a fundamental role in testing novel antiepileptic drugs (AEDs) and helped in determining the pathological and physiological pathways associated with human epilepsy [5]. Pentylenetetrazol (PTZ) is a convulsant chemical agent that has been frequently used in experimental models for seizure induction [6,7]. This noncompetitive antagonist blocks GABA-mediated Cl^- influx through an allosteric interaction in the Cl^- channel, thus leading to the depolarization of neuronal membrane and propagation and maintenance of seizure activities. Acute effects of PTZ are largely mediated by its action in the GABAA receptors [8–10]. Nevertheless, some contributions by other receptors have also been made. Discriminative stimulus effects of PTZ can be modulated by nicotinic [11,12] and NMDA [13] receptors and brain monoaminergic systems such as serotonin and dopamine [14–17]. Previous reports have indicated the critical role of brain monoamine in establishing the convulsion threshold [18]. It has also been shown that exposure to PTZ increases the density of metabotropic glutamate receptor and activity of the opioid system and that it causes a number of biologic alterations in the hippocampus including inositol triphosphate formation in PTZ-kindled rats [19,20].

Morphine use and abuse can also alter seizure threshold [21,22]. However, the activity of the opiate system influences the expression of seizures in contrasting ways, depending on opiate dose and mode of seizure induction [23]. Morphine can modulate seizure susceptibility in a biphasic manner [24,25] and cause dose-dependent anticonvulsant and proconvulsant effects. Intracranial administration of morphine and opioid peptides elicits pathologic epileptiform activity in the

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electroencephalogram [26] and may induce seizures in humans [27]. A potential withdrawal condition caused by the abrupt discontinuation of opiate intake after an extended period of abuse may also induce seizure-like activity [28].

Tramadol hydrochloride, which is widely used throughout the world, is a centrally acting analgesic prescribed for moderate-to-severe pain [29,30]. Tramadol and morphine bind to μ-opioid receptors; however, tramadol has several-time weaker affinity with this receptor than morphine [30]. Tramadol does not precipitate withdrawal symptoms [31]. Several reports and controlled laboratory studies have indicated that it may be effective in relieving opioid withdrawal symptoms [32,33]. Despite its affinity with the opiate receptor, tramadol is not chemically related to opiates [34]. However, its effects are attributed to its opioid and nonopioid (inhibition of noradrenaline and serotonin reuptake) actions [35,36]. In preclinical evaluation, tramadol displays both proconvulsant and anticonvulsant properties [37–39]. Some studies have indicated that tramadol can only provoke seizures if used in excessive doses in patients with epilepsy or if coadministered with other seizure-inducing drugs [40,41]. Because of tramadol's dual mechanism of action, its adverse effect profile can be different in comparison with single-mechanism opioid analgesics, such as morphine. Despite numerous studies of the dose-dependent and biphasic effects of tramadol and morphine on seizures, there is still lack of knowledge about the comparison of specific effects of morphine and tramadol on seizures, especially PTZ-induced seizures, following acute and chronic administration or withdrawal using different methods of administration (IP or PO). On the other hand, because multiple receptor systems are involved in triggering opioid-induced seizures, such as opioid, adrenergic, glutamatergic [42], and opioid antagonism of inhibitory GABA neurotransmission [43], it seems that there are some interactions between PTZ- and opioid-induced seizures. Therefore, in the present study, PTZ-induced seizure was used as a model in order to determine whether administration (or withdrawal) of morphine and tramadol would augment PTZ-induced seizures in rats or not.

2. Materials and methods

2.1. Animals

Adult female Wistar rats (n = 72) weighing 180–200 g were housed in Plexiglas cages in a colony and maintained at 22 ± 2 °C with a 12-h light/dark cycle (lights were turned on at 0700). These rats were allowed free access to food and water. Bedding consisted of untreated wood shavings and was changed three times a week. All the experimental protocols and procedures were in agreement with the guidelines of the 1975 Declaration of Helsinki, as reflected in the guidelines of Medical Ethics Committee, Ministry of Health, Iran. In addition, Regional Medical Ethics Committee of West Azerbaijan Province, Islamic Republic of Iran, approved this study.

2.2. Drug administration

The rats were randomly divided into three groups for either intraperitoneal (IP) or gavage (PO) administrations by saline, morphine (Temad Co., Tehran, Iran), and tramadol (Atlantis Life Sciences Co., Mumbai, India). Rats in morphine and tramadol groups received morphine or tramadol with an increasing dose (20, 27.5, 35, 37.5, 45 …, until 125 mg/kg) once per day for 15 consecutive days. Administered dose of morphine/tramadol was increased by 7.5 mg/kg every day. These increasing doses of tramadol (IP) have been previously used in different studies [44]. The saline group received saline in a similar manner. Following one week of withdrawal period, the rats in all the groups were further divided into subgroups on the 22nd day of the experiment. In the saline (IP and PO) groups, a total number of 30 rats were divided into 3 subgroups which received saline [saline/saline = SS: SS IP (n = 5) and SS PO (n = 5)], morphine [saline/morphine = SM: SM IP (n = 5) and SM PO (n = 5)], or tramadol [saline/tramadol = ST: ST IP (n = 5) and ST PO (n = 5)]. In tramadol groups, a total number of 22 rats were divided into 2 subgroups which received saline [tramadol/saline = TS: TS IP (n = 5) and TS PO (n = 5) or tramadol [tramadol/tramadol = TT: TT IP (n = 7) and TT PO (n = 5)]. Finally, in morphine groups, 20 rats were divided into 2 subgroups which received saline [morphine/saline = MS: MS IP (n = 5) and MS PO (n = 5) or morphine [morphine/morphine = MM: MM IP (n = 5) and MM PO (n = 5)]. Rats in SM and ST groups were considered to have acute drug administration because they were not exposed to morphine or tramadol during 15 days of the treatment. Similarly, those in MS and TS groups were considered to have chronic drug administration because of continuously receiving an increasing dose of morphine or tramadol for 15 days to establish the dependence model. Rats in MM and TT groups, with one week of withdrawal period, were considered to present a model of chronic use, withdrawal, and relapse. Rats of all the groups received PTZ (80 mg/kg, IP) 30 min after saline, morphine, or tramadol administration. All morphine, tramadol, and saline administrations occurred at the same time each day (1100 and 1200). Morphine was dissolved in 0.9% saline and freshly prepared for each use; however, tramadol hydrochloride was in the liquid form. Effects of drug administration on PTZ-induced seizure behaviors were investigated one week later because the previous study of the present authors showed that the increasing doses of morphine and tramadol in a neonatal period had a long-term effect (a week later) on PTZ-induced seizures [45]. To avoid different estrous cycles in rats and minimize effect of sex hormone fluctuations on their behavior [46], all the subjected rats were arranged to be in metestrous period when the experiment was started [47]. The rats were gently held in the hand, and a vaginal smear was obtained in the morning (9 h). Sterile cotton-tipped swabs wetted in distilled water were gently introduced into the vaginal orifice; the introduction was relatively shallow (approximately 1 cm) to avoid excessive cervical stimulation and a consequent pseudopregnancy. Subsequently, they were carefully rotated (one twist) against the vaginal wall [48]. Rats were not anesthetized during smear collection. The vaginal smear was mounted on a glass slide with a cover slip and was observed under light microscopy. The metesterous period was identified with high number of leukocytes as well as few nucleated epithelial cells [48,49]. If a rat was not in the metestrous period, she was returned to her home cage and was retested 1–3 days later according to the result of her vaginal smear. However, it is likely that the females may not have been selected exactly at the same cycle stage because the method used here was not very accurate in distinguishing the cycle stage. We avoided collecting vaginal smears of the females for four consecutive days to minimize handling-induced stress in subjects.

2.3. Apparatus

The seizure test cage was a round, plywood cage 81.5 cm in diameter, closed by a wall which was 31.5 cm high. The light source was a 150-W lamp, hanging 1.20 m above the floor level, similar to the one used by Martin-Garcia and Pallares [50].

2.4. Seizure testing

Rats were transferred to the testing room one day before the experiment to be acclimatized to the new environment. A seizure was induced by the IP injection of PTZ (80 mg/kg) [51,52] on the 22nd day of the experiment. Immediately after the injection, the rats were individually placed in the center of the apparatus, and their behaviors were videotaped and monitored for 40 min. They were then tested in a random order. The seizure test cage was cleaned at the end of each trial to prevent behavioral modifications due to the presence of odor. All the rats were seizure-naive when tested, and each one was subjected