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Effects of Ethylketocyclazocine on Behaviors in Mice Using Multi-Dimensional Behavioral Analyses

MAKOTO UKAI AND TSUTOMU KAMEYAMA[1]

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences
Meijo University, Nagoya 468, Japan

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UKAI, M. AND T. KAMEYAMA. *Effects of ethylketocyclazocine on behaviors in mice using multi-dimensional behavioral analyses.* PHARMACOL BIOCHEM BEHAV **28**(1) 43–46, 1987.—The effects of ethylketocyclazocine, a relatively selective kappa opioid agonist on the behaviors in mice were examined by using multi-dimensional behavioral analyses. Within 15 min after the measurements, ethylketocyclazocine (0.03, 0.1 and 0.3 mg/kg) dose-dependently decreased the linear locomotion, rearing, and grooming behaviors in mice. Fifteen to 30 min ethylketocyclazocine (0.3 mg/kg) decreased most of the behaviors in mice. The ethylketocyclazocine (0.3 mg/kg)-induced behavioral effects were antagonized by naloxone (1 and 2 mg/kg). These data suggest that the decreases in the linear locomotion, rearing and grooming behaviors are mediated via kappa opioid system.

Ethylketocyclazocine Naloxone Locomotor activity Mice

MARTIN *et al.* [10] have provided the first in vivo pharmacological evidence for multiplicity of opiate receptors. It has been demonstrated that the subtypes of opioid receptors such as mu, kappa, sigma and delta exist in the mammalian brains [3, 4, 12]. Morphine is a representative ligand for the mu receptors. Ethylketocyclazocine, a benzomorphan, is one of the prototypic kappa agonists. SKF 10,047 (N-allylnormetazocine), a potent hallucinogen in man and an effective morphine antagonist with little analgesic activity is a prototypic sigma agonist. According to recent studies, morphine and SKF 10,047 elicit hypermotility, whereas ethylketocyclazocine induces sedation in mice [3].

On the contrary, there is a large body of evidence regarding the effects of opioid peptides on the behaviors in mice [5–7, 13–17]. It has been shown that α-, β- and γ-endorphins enhance the linear locomotion in mice by using multi-dimensional behavioral analyses [5,6]. In a subsequent study, the behavioral effects of the above peptides parallel with the alteration of dopamine turnover in the striatum of mice [7]. More recently, it has been reported that dynorphins are specific endogenous ligands of the kappa opioid receptors [1,2]. Nevertheless, there exist clear behavioral differences between dynorphin-(1–13) [D13A] and dynorphin A[dynorphin-(1–17)] in mice [14,16]. The former increases the linear locomotion, while the latter decreases it. Although the behavioral differences between dynorphins would be due to the length of the peptide fragments, the contribution of kappa opioid receptor stimulation to the behavioral effects of both dynorphins still remains inconclusive.

To provide more accurate information about the role of kappa opioid receptors in the behaviors in mice, the current study aimed at clarifying the effects of the relatively specific kappa agonist ethylketocyclazocine on behaviors in mice by using multi-dimensional behavioral analyses. Additionally, the effects of naloxone on the ethylketocyclazocine-induced behaviors were examined to confirm whether or not they were mediated by opioid receptors.

METHOD

Male ddY mice (Shizuoka Experimental Animal Agricultural Cooperative Association, Japan) weighing between 18 and 25 g were used in the experiments. The animals arrived at least 3 days before the experiments. Food and water were available ad lib inside the cage in a constantly illuminated room at a temperature of 23±1°C and a relative humidity 55±2.5%. Ethylketocyclazocine methanesulfonate (Sterling-Winthrop Research Institute, NY) was suspended in 0.3% carboxymethylcellulose solution containing 0.9% isotonic saline. Naloxone hydrochloride (DuPont Pharmaceuticals, PA) was dissolved in 0.9% isotonic saline. Doses refer to the salts.

Behaviors were measured for 30 min. Ethylketocyclazocine and naloxone were administered 10 and 15 min before the measurements, respectively. The Animex II (LKB-Farad, Sweden) equipped with an electronic microcomputer, was used for the measurements. The sensor consisted of three pairs of electrodes and formed a capacitor bridge. Once

[1]Requests for reprints should be addressed to T. Kameyama.

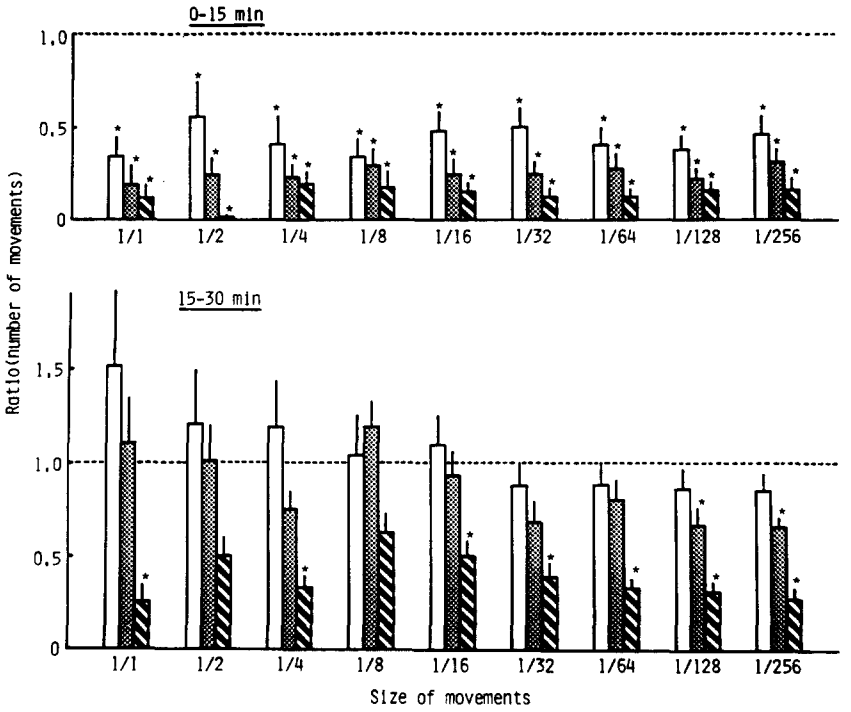

FIG. 1. Spontaneous movements in mice after ethylketocyclazocine. Values depict the mean±S.E. for 10 mice. *Denotes significant difference from control, $p<0.05$. Dashed line: control, open column: ethylketocyclazocine 0.03 mg/kg, SC, dotted column: 0.1 mg/kg, SC, striped column: 0.3 mg/kg, SC.

a mouse was placed in the space (150×210×140 mm) among the electrodes, the values of the capacitor then depended on the location of the mouse within that space. After converting the analog signal to a digital form, the DC-voltage movement spectrum analyser classified the movement into nine degrees (1/1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128 and 1/256). Since the surface areas of the cage in which mice could behave (ambulation, rearing and circling) was 490 mm, a 1/1 size of movement meant a series of 490 mm distance run. Particularly, the 490 [210+(140×2)] mm distance consisted of the length of the cage bottom and cage walls. Thus, the counters corresponded to the following movement sizes, 1/1=490 mm, 1/2=245 mm, 1/4=123 mm, 1/8=61 mm, 1/16=31 mm, 1/32=15 mm, 1/64=8 mm, 1/128=4 mm and 1/256=2 mm. The greatest movement was primarily registered on the 1/1 counter, and the smallest movement, such as tremor, on the 1/256 counter. Specific behavioral patterns induced by a drug were registered on the counters as follows, linear locomotion on 1/1 and 1/2, rearing and circling on 1/4 and 1/8, grooming on 1/16, 1/32 and 1/64, and sniffing and convulsion on 1/128. The sensitivity (%) of the device was adjusted according to the body weight (g) as follows, 18 g=29%, 19 g=28%, 20–21 g=27%, 22–23 g=26% and 24–25 g=25%.

The ordinate in the figures was labeled "ratio (number of movements)=(value of drug-treated animal)/(mean value of controls)."

Data were statistically analysed by means of a one-factor analysis of variance (ANOVA). Post-hoc analysis for between group differences was carried out by employing the Newman-Keuls method for multiple comparisons. A p value of less than 0.05 was taken as the level of statistical significance.

RESULTS

Effects of Ethylketocyclazocine on Movement Spectrum

Within 15 min after the measurements, ethylketocyclazocine (0.03, 0.1 and 0.3 mg/kg) significantly inhibited the linear locomotion, rearing and grooming behaviors in mice (Fig. 1). The ethylketocyclazocine (0.03 mg/kg)-induced decrease in the behaviors disappeared between 15 and 30 min after the measurements. A 0.3 mg/kg dose produced marked decreases in the linear locomotion, rearing and grooming behaviors even in the latter half of observations. The ethylketocyclazocine (0.3 mg/kg)-induced decreases in the behaviors were almost completely abolished by naloxone (1 and 2 mg/kg) (Fig. 2).

DISCUSSION

It has been demonstrated that unlabeled ethylketocyclazocine displaces mu_2(^{3}H-dihydroxymorphine) and delta (^{3}H-d-ala^2-d-leu^5-enkephalin) opioid receptor sites as potently as it displaces ^{3}H-ethylketocyclazocine binding [3]. However, the poor ability of unlabeled morphine and d-ala^2-d-leu^5-enkephalin to inhibit ^{3}H-ethylketocyclazocine binding [3] shows that ^{3}H-ethylketocyclazocine does bind to an additional site other than mu_2 or delta. Kappa opioid receptor sites have been reported to be rather specific for benzomorphans such as ethylketocyclazocine and ketocyclazocine [4,12].

Ethylketocyclazocine as a representative of kappa opioid agonist [3, 4, 12] produces potent analgesia [19] as well as increases in food and water intake in rodents [8,9]. The present data indicate that ethylketocyclazocine produced a

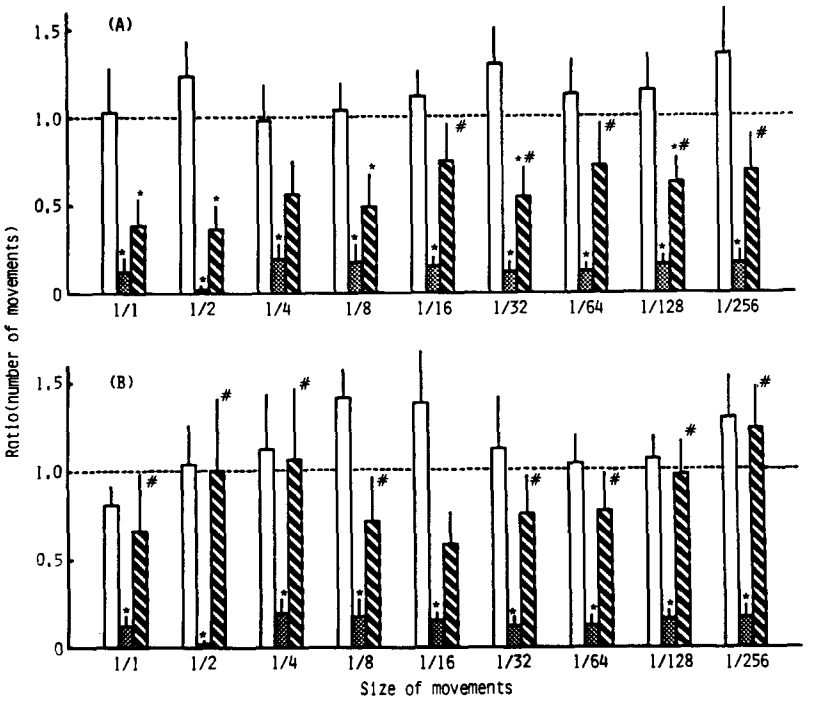

FIG. 2. Spontaneous movements in mice within 15 min after measurements of ethylketocyclazocine-induced behavior and the influence of naloxone. Values depict the mean±S.E. for 10 mice. (A) Dashed line: control, open column: naloxone 1 mg/kg, SC, dotted column: ethylketocyclazocine 0.3 mg/kg, SC, striped column: naloxone 1 mg/kg, SC + ethylketocyclazocine 0.3 mg/kg, SC. (B) Dashed line: control, open column: naloxone 2 mg/kg, SC, dotted column: ethylketocyclazocine 0.3 mg/kg, SC, striped column: naloxone 2 mg/kg, SC + ethylketocyclazocine 0.3 mg/kg, SC. *Denotes significant difference from control, $p<0.05$. #Denotes significant difference from ethylketocyclazocine (0.3 mg/kg), $p<0.05$.

naloxone-reversible decrease in the linear locomotion, rearing and grooming behaviors by using multi-dimensional behavioral analyses, thus suggesting that the behaviors induced by ethylketocyclazocine are mediated via kappa opioid scheme. Similar evidence has been reported in the behavioral effects of U-50,488H, a recently-developed kappa agonist [18]. In contrast, we have previously reported that the intracerebral injection of morphine (20 and 40 μg), a selective mu opioid agonist, elicits naloxone-reversible circling behaviors [6].

Inasmuch as dynorphins have high affinity for kappa opioid receptors in the brain, they are considered to be endogenous kappa agonists [1,2]. Nevertheless, the behavioral effects of each of D13A and dynorphin A are different. For example, D13A significantly increases the linear locomotion, while dynorphin A markedly decreases most of the behaviors in mice by using multi-dimensional behavioral analyses [14,16]. The difference between the behavioral effects of D13A and dynorphin A may be due to the degrees of opioid receptor preference. The behavioral effects of ethylketocyclazocine resembled those of U-50,488H and dynorphin A unlike D13A. It thus appears that ethylketocyclazocine, U-50,488H, and dynorphin A with a longer fragment than D13A display kappa opioid receptor-mediated activity. This consideration would be strengthened by the evidence reported by Shultz *et al.* [11] in which they demonstrate kappa receptor activity for the longer fragments and delta activity for the shorter fragments of dynorphins in the tolerance studies of mouse vas deferens.

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