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Effects of a Kappa Receptor Agonist, Ethylketocyclazocine, on Water Consumption in Water-Deprived and Nondeprived Rats in Diurnal and Nocturnal Tests

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TURKISH, S. AND S. J. COOPER. *Effects of a kappa receptor agonist, ethylketocyclazocine, on water consumption in water-deprived and nondeprived rats in diurnal and nocturnal tests.* PHARMACOL BIOCHEM BEHAV **21**(1) 47–51, 1984.—In 24 hr water-deprived male hooded rats, ethylketocyclazocine (EKC), 0.1–3.0 mg/kg, dose-dependently suppressed water intake. Within the first 30 min access to water, drinking was virtually abolished by 1.0 and 3.0 mg/kg EKC. Significant reductions in the level of water intake were found after 0.1 mg/kg EKC. After 2 hr access to water, the suppressant effect was attenuated indicating some recovery. The antidipsogenic action of EKC in water-deprived rats was comparable in its effect for both daytime and nocturnal testing. Circadian variation may not be an important modulator of the antidipsogenic action. Naloxone, an opiate receptor antagonist, when administered in a dose of 0.3 mg/kg also significantly reduced drinking in deprived animals. EKC (0.3 mg/kg) and naloxone (0.3 mg/kg) when administered together displayed mutual antagonism. Drinking was at control levels. In nondeprived male rats, EKC exerted some dipsogenic action, most noticeably during diurnal testing. Within 30 min access to water, 0.1 mg/kg EKC significantly elevated the level of water intake. This effect did not occur during nocturnal testing, when the only immediate effect of EKC was a suppression of drinking at 1.0 and 3.0 mg/kg dose levels. After 2 hr access to water, there was a significant peak effect to enhance drinking at the 0.3 mg/kg dose level during the daytime. Effects of EKC during the night were less pronounced. The dipsogenic action of EKC (0.3 and 1.0 mg/kg) in satiated animals during the day was abolished by naloxone and Mr-2266BS, also an opiate receptor antagonist. The results indicate that the effects of EKC on drinking are profoundly affected by the deprivation status of the animal. Further, the phase of the circadian cycle may be an important modulator of the expression of the dipsogenic action of EKC in nondeprived rats.

Circadian cycle Endorphins Ethylketocyclazocine Kappa receptor Mr-2266BS Naloxone Thirst Water consumption Water-deprivation Rats

ONE of the most interesting findings to emerge in the analysis of the effects of opiate receptor agonists and antagonists on feeding and drinking [15] is the powerful modulating influence of deprivation condition. In two experiments, Sanger and McCarthy showed first that morphine (1.0–30.0 mg/kg) depressed levels of food and water intake in 24 hr food-deprived rats; second that morphine (over the same dose range) increased levels of food and water intake in nondeprived rats [16]. In a later study, the same authors confirmed that in nondeprived rats, the mu opiate receptor agonist RX 78030 (0.03–10.0 mg/kg) and the kappa opiate receptor agonist ethylketocyclazocine (EKC, 0.1–10.0 mg/kg) increased food and water intake over a 6 hr period [17]. The effects of RX 78030 and EKC on water intake appeared to be less reliable than their actions on feeding, and to occur later than the effects on feeding. Sanger and McCarthy were not clear, therefore, whether the increases in water intake occurred as secondary responses to the elevation in food intake [17]. On the other hand, we have observed increases in water intake without change in food intake following the administration of morphine to nondeprived rats and pigeons [1,2].

We decided therefore to investigate the effects of the kappa agonist EKC [5, 14, 22] on water consumption in the absence of food, to determine whether it would stimulate water consumption directly. Secondly, we wished to determine whether the action of EKC on drinking would be modulated by the deprivation state of the animal. Thirdly, we

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were interested to know whether the actions of EKC were modified to any degree by testing in the nocturnal phase as compared to the diurnal phase of the daily light-dark cycle. There is evidence for circadian variation in endogenous opioid peptide levels and activity [3,13]. In sum, we wished to determine if deprivation status and circadian variation were potent modulators of the actions of EKC on water ingestion.

METHOD

Animals

The subjects were 100 experimentally naive adult male rats (hooded General strain) which were bred in the animal laboratory of the Psychology department. They were housed individually in stainless steel cages with continuous access to standard laboratory food pellets (small animal diets, Pillbury's Ltd.) and tap water. They were kept in a laboratory where room temperature was maintained at 21–22°C. Room illumination operated on a 12 hr light–12 hr dark cycle, with light on at 07.00 hr. The rats weighed between 200 and 300 g at the start of testing.

Procedure

The drinking tests were conducted in the animals' home cages. Water was presented in a calibrated cylinder which was clipped to the front of a cage, with the metal spout protruding into the interior. Water consumption was measured to the nearest 1.0 ml by reading the level in the water container. During drinking tests, food was removed from the home cage.

The animals were divided into two equal groups. One set of animals were 24 hr water-deprived prior to the drinking test, and the other set were tested in a nondeprived condition. During the week before the drinking tests, the water-deprivation group were 24 hr water-deprived every 2 days to ensure stabilised baseline drinking patterns. All animals were accustomed to handling and received vehicle injections to become familiarised with the injection procedure.

Each batch of 50 animals was further divided into 5 equal subgroups. Water consumption (ml) was measured after the administration of a control injection (distilled water) respectively. Doses refer to the salt, injections were administered subcutaneously in a volume of 1 mg/kg, and distilled water was used as the solvent.

In the first study, all animals were tested during the diurnal phase of the daily light-dark cycle (11.30–13.30 hr). EKC was injected 30 min prior to the presentation of water in the calibrated cylinder. Water consumption was measured at 30 min, 1 hr and 2 hr after the start of the drinking test.

One week later, all animals were tested on a second occasion, but during the nocturnal phase of the daily light-dark cycle. The period 19.30–21.30 hr was chosen because rats show increased nocturnal activity especially at the beginning of the dark phase (Cooper, Burton and Popplewell, unpublished videorecording data). In this second study, animals that had previously been tested nondeprived were tested after 24 hr water-deprivation. Those that had previously been deprived were tested under the nondeprivation condition. The water-deprived animals were habituated to 24 hr water deprivation every second day. Injection and test conditions were the same as those used in the daytime study.

Opiate antagonism studies were also carried out, in attempts to reverse the effects of EKC. In the first study, 70 animals were selected at random from the larger group of animals used previously, and these were divided into 7 equal groups. Animals were tested nondeprived during the daytime test period. Each animal received two injections prior to the start of the drinking test. In 3 groups the first injection was 0.3 mg/kg EKC, in 3 groups the first injection was distilled water vehicle, and in 1 group the injection was 1.0 mg/kg EKC. These injections were administered subcutaneously 30 min before the start of the drinking test. The second injection was administered subcutaneously 15 min later. In the 3 groups which had received 0.3 mg/kg EKC, the second injection was distilled water, 0.3 mg/kg naloxone hydrochloride and 0.3 mg/kg Mr-2266 BS, [(−)-2-(3-furyl-methyl)-5, 9-diethyl-2-hydroxy-6,7-benzomorphan], respectively. In the 3 groups injected with distilled water first, the second injection was distilled water, 0.3 mg/kg naloxone HCl and 0.3 mg/kg Mr-2266BS, respectively. Animals injected with 1.0 mg/kg EKC first were given a second injection of distilled water. Mr-2266BS has been reported to be more potent than naloxone in antagonizing effects mediated by kappa receptor activation, whereas naloxone is more potent than Mr-2266BS in antagonizing effects mediated by mu receptor activation [14,18]. Doses of naloxone and EKC refer to the salts, which were dissolved in distilled water. Mr-2266BS is a base, and was dissolved in distilled water with the aid of a few drops of hydrochloric acid and gentle warming. Water consumption (ml) was determined at 30 min and 2 hr after access to water was given.

In the second drug antagonism study, 60 of the animals were selected at random and were divided into 6 equal groups. Animals were tested 24 hr water-deprived during the diurnal test period. Each animals received two injections before the start of the drinking test. Three groups received a first injection of EKC (0.3 mg/kg) and 3 groups received a control injection of distilled water, administered 30 min before the drinking test. The second injection was administered 15 min later, and consisted of distilled water, 0.1 mg/kg or 0.3 mg/kg naloxone respectively, within each of the 3 groups. All injections were given subcutaneously. Water consumption (ml) was measured at 30 min and 2 hr after access to water was given.

Drinking data were analysed using analysis of variance procedures and by Dunnett's t-test to determine significant differences from control values for individual drug treatment conditions [21].

RESULTS

Nondeprived Animals

The effects of EKC (0.1–3.0 mg/kg) on water consumption (ml) in nondeprived rats are shown for light and dark periods in Fig. 1. After only 30 min access to water, EKC significantly elevated the level of water intake when given in a small dose of 0.1 mg/kg, during daytime testing. Drinking was suppressed at the highest dose tested, 3.0 mg/kg. EKC did not stimulate water consumption during this first 30 min period during nocturnal testing. The only significant effects were complete suppression of drinking when EKC was given in doses of 1.0 and 3.0 mg/kg.

After 2 hr access to water, EKC showed a clear nonmonotonic effect on water consumption. Water intake was elevated following the administration of EKC in light and dark phases, with the peak effect occurring at 0.3 mg/kg. No enhancement of drinking took place at 3.0 mg/kg EKC. As Fig. 1 indicates, more drinking occurred following EKC administration during the daytime test.

Significant hyperdipsic effects of EKC (0.3 and 1.0 mg/kg)

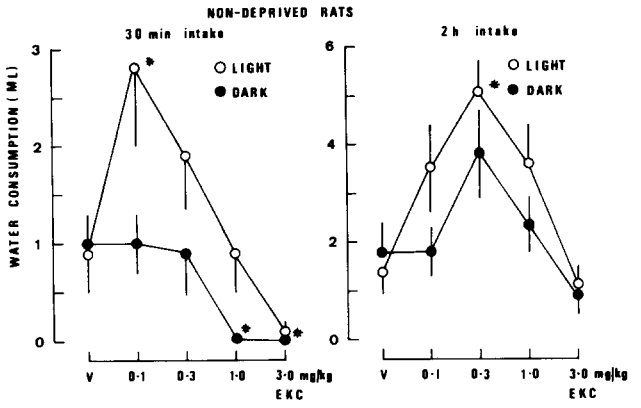

FIG. 1. Effects of EKC (0.1–3.0 mg/kg) on water consumption of satiated male rats observed during diurnal (○) and nocturnal (●) phases. Intakes after 30 min access to water are shown in the left panel, and after 2 hr access in the right panel. Each data point represents the mean of 10 animals. Vertical lines indicate SEMs. *$p<0.05$ (Dunnett's test): comparison between drug treatment and corresponding vehicle (V) control condition.

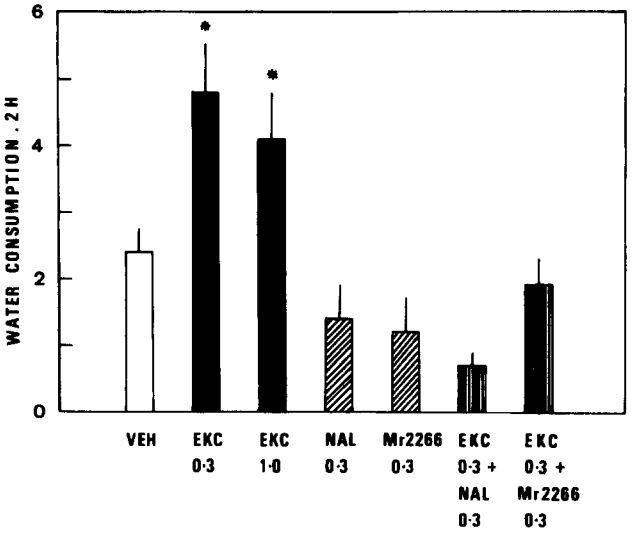

FIG. 2. Hyperdipsic effects of EKC (0.3 and 1.0 mg/kg) in satiated rats over 2 hr access to water in the daytime. Opiate receptor antagonists naloxone (0.3 mg/kg) and Mr-2266-BS (0.3 mg/kg) completely block the hyperdipsia. Histogram bars represent the mean results for separate groups of 10 animals. Vertical lines indicate SEMs. *$p<0.05$ (Dunnett's test): comparison between drug treatment and corresponding vehicle (VEH) control condition.

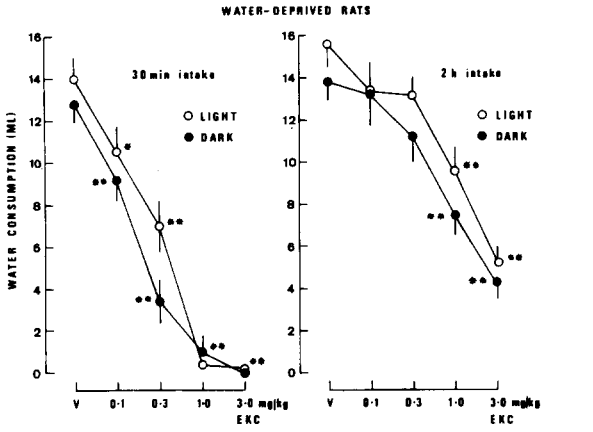

FIG. 3. Antidipsogenic effects of EKC (0.1–3.0 mg/kg) in 24 hr water-deprived rats measured during diurnal and nocturnal phases. For other details see legend to Fig. 1. **$p<0.01$ (Dunnett's test).

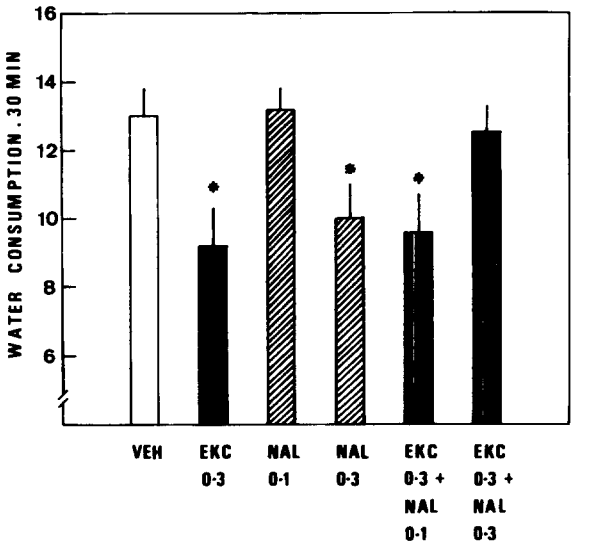

FIG. 4. Antidipsogenic effects of 0.3 mg/kg EKC and 0.3 mg/kg naloxone in 24 hr water-deprived rats over 30 min access to water in the daytime. The two drugs in combination showed mutual antagonism, so that water intake remained at a control level. For other details see legend to Fig. 2.

over a 2 hr period in the light phase were completely abolished by the opiate receptor antagonists naloxone (0.3 mg/kg) and Mr-2266BS (0.3 mg/kg) (Fig. 2).

Water-Deprived Animals

EKC produced dose-dependent decreases in water intake in 24 hr water-deprived rats (Fig. 3). After 30 min access to water, control levels of water intake were 13.9±1.35 ml (mean±SEM) during the light and 12.8±0.93 ml during the dark. Drinking was virtually completely suppressed by EKC in doses of 1.0 and 3.0 mg/kg. Significant reductions in drinking were obtained when EKC was administered in a dose of 0.1 mg/kg. After 2 hr access to water, there appeared to be some recovery from the antidipsogenic effects of EKC treatments. Significant reductions in water consumption remained in the 1.0 and 3.0 mg/kg groups, but drinking was no longer completely suppressed. Analysis of variance showed no significant overall main effect of light/dark phase on drinking in the deprived animals, and no significant interaction with EKC treatments. EKC suppressed water consumption equivalently during diurnal and nocturnal tests.

The significant antidipsogenic effect of EKC (0.3 mg/kg) in the first 30 min access to water in the light phase was completely reversed by naloxone in a dose of 0.3 mg/kg, but not in a dose of 0.1 mg/kg (Fig. 4). Interestingly, naloxone (0.3 mg/kg) itself produced a significant reduction in the level of water intake. The effects of EKC (0.3 mg/kg) and naloxone (0.3 mg/kg) were clearly mutually antagonistic.

DISCUSSION

EKC, a kappa receptor agonist, dose-dependently reduced the level of water intake in 24 hr water-deprived rats. Complete suppression of drinking in the first 30 min access to water occurred following the administration of higher doses, 1.0 and 3.0 mg/kg. The reduction was less marked after 2 hr access, indicating possible recovery from antidipsogenic effects of EKC. There were no differences between the effects of EKC assessed by day and during the nocturnal period. The antidipsogenic effect of 0.3 mg/kg EKC were fully reversed by the opiate receptor antagonist, naloxone, consistent with opiate-receptor mediation of the affect. This result is consistent with the report that bremazocine, a kappa receptor agonist [14], dose-dependently suppressed 2 hr water consumption in 22 hr water-deprived rats [8]. On the other hand, a further study reported that bremazocine selectively enhanced intake over a 12 hr nocturnal period in 12 hr food- and water-deprived female rats [4]. The difference between results is probably due to the different periods over which water consumption was measured. In the short term, we have demonstrated a powerful suppression of drinking produced by EKC in 24 hr water-deprived animals within 30 min access to water. It is possible that the enhanced water consumption observed by Hartig and Opitz [4] may have been secondary to marked water loss as a result of the suppression of vasopressin release [8].

During diurnal testing, small doses of EKC (0.3 and 1.0 mg/kg) significantly enhanced water consumption in satiated rats over a 2 hr period (Figs. 1 and 2). Within 30 min access to water, a dose as small as 0.1 mg/kg EKC was sufficient to significantly stimulate water consumption. The daytime hyperdipsic effect of EKC was reversible by naloxone and Mr-2266BS. During nocturnal testing EKC had no hyperdipsic effect in the first 30 min access to water; instead drinking was significantly suppressed at 1.0 and 3.0 mg/kg. Testing during the dark phase attenuated the hyperdipsic effect of EKC measured over 2 hr of the drinking test (Fig. 1).

These results suggest that the hyperdipsic effect of EKC in nondeprived rats observed previously [17] was not necessarily a secondary effect arising from EKC's stimulation of food intake. However, there are several reports that in nondeprived animals kappa agonists have a marked diuretic effect which is due to a suppression of vasopressin release [6, 7, 8, 12, 19, 20]. One plausible explanation of the hyperdipsic effect of EKC is therefore one which emphasises a polyuria followed by a compensatory hyperdipsia. In support of this interpretation, an inverted U-shaped dose-effect curve on 2 hr urine output has been reported [6] which agrees with the 2 hr water intake results we obtained (Fig. 1). Further research is required to establish whether or not the control of vasopressin release by actions at kappa receptors is responsible for the observed effects of kappa agonists and antagonists on drinking ([6, 7, 8, 9, 12, 17, 19, 20], present study).

Recently we have observed that EKC (0.1–3.0 mg/kg, SC) affected the consumption of palatable wet mash in nondeprived rats (Jackson and Cooper, unpublished data). General behavioral depression was evident within the first hour after administration of the higher doses of EKC. It is therefore probable that the dose-dependent reduction in drinking we observed in thirsty rats, in which the baseline water consumption was high, can be accounted for in terms of nonspecific behavioral suppression. Hence our results with EKC, in nondeprived and in water-deprived rats, do not require direct effects of EKC on the controls of drinking. The hyperdipsia in nondeprived rats and the suppression of drinking in deprived animals may have reflected a diuretic effect and a general suppressant effect of EKC, respectively. Nevertheless, the possibility that there is a direct involvement of kappa receptors in the controls of drinking should not, at this stage, be entirely ruled out.

Our behavioral results with EKC may bear some relationship to recent biochemical data. Reid and his colleagues report immunoreactive beta-endorphin and dynorphin in rat brain and pituitary after food and/or water deprivation, and from brains taken during either day or night [13]. There is growing evidence that dynorphin is the endogenous ligand for the kappa receptor in brain [10,11]. Apparently, water deprivation leads to increased immunoreactive dynorphin in rat hypothalamus and to markedly decreased immunoreactive dynorphin in the neurointermediate lobe of the pituitary [13]. Circadian variation in dynorphin levels also occur. Dynorphin levels were lower in hypothalamus during the day compared to the night. The opposite was true for dynorphin in the neurointermediate lobe of the pituitary [13].

Our present data indicated that EKC produced a hyperdipsic effect in nondeprived animals which was more prominent during the light phase. If this effect did reflect an action of EKC to suppress vasopressin release, then we should expect also that the effectiveness of this suppression may be influenced by the phase of the light-dark cycle. The biochemical evidence, cited above, indicated that there are circadian changes in brain and pituitary dynorphin levels. The constant suppression of water consumption by EKC in water-deprived rats, irrespective of light- or dark-phase testing indicates that this effect may be little affected by circadian change in endogenous opioid peptide levels.

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