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Dapiprazol Prevents U50,488H-Mediated Suppression of Preparatory Components of Drinking Behavior in Rats

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NENCINI, P., M. GRAZIANI AND P. VALERI. *Dapiprazol prevents U50,488H-mediated suppression of preparatory components of drinking behavior in rats.* PHARMACOL BIOCHEM BEHAV **40**(1) 125–128, 1991.—In a previous study, we found that the kappa opioid agonist U50,488H (U50) suppresses both appetitive and consummatory components of drinking behavior in rats trained to negotiate water in a straight runway. U50 also activates diuresis. Kappa opioid mechanisms could therefore play a dissipative role in the body's water balance. Since naloxone inhibits diuresis, but not hypodipsia produced by U50, these effects are probably mediated also by nonopioid mechanisms. In rats trained to negotiate water in a straight runway, we have studied the influence on the hypodipsic effects of U50 of the selective alpha-1 adrenoceptor antagonist dapiprazol (DAP), which we found to inhibit U50-mediated diuresis. When given alone, DAP (3 and 6 mg/kg IP) influenced neither running for water nor water intake; neither did it prevent the suppression of water intake produced by U50 (8 mg/kg IP) across the test. However, it did antagonize the U50-mediated slowing of running for water. Alpha-1 adrenoceptors thus appear to play a role in U50's effects on diuresis and the appetitive, but not consummatory, aspects of drinking.

Drinking Runway U50,488H Kappa receptors Dapiprazol Alpha-1 adrenoceptors Rat

SYSTEMATIC study of the pharmacological effects of selective kappa opiate agonists shows that these drugs strongly activate feeding and diuresis [for reviews, see: (3,4)]. Although feeding and diuresis are clearly mediated by mechanisms that are physiologically independent, they have in common the capability of activating drinking behavior. Thus kappa opiate agonists are expected to stimulate water intake. However, experimental evidence shows that kappa opiate agonists produce either an increase or a decrease in drinking, according to the circumstances (3). In particular, food presentation appears to influence the drinking response to kappa opiate agonists. In water-deprived rats without access to food during drinking session, intracerebroventricular injection of dynorphin$_{1-13}$ strongly suppresses drinking (13). However, Ukai and Holtzman (15), administering the same peptide intrahypothalamically to hydrated or water-deprived rats that had no access to food during drinking sessions, found no change in water intake. In contrast, dynorphin A_{1-13} injected into the preoptic area of rats with access to food during the experimental session, has been found to stimulate water intake (12). On the whole, these studies suggest that the primary effect of kappa opiate agonists on water intake is inhibition, but that this action may be concealed by prandial drinking. Consistent with this hypothesis is that the appetitive (i.e., running for water) and consummatory components of drinking behavior are both suppressed by U50,488H (U50), a selective kappa opiate agonist, in rats trained to negotiate water in a runway in absence of food (8).

The influence of the diuretic effects of kappa opiate agonists on their actions on water intake is harder to understand. Since diuresis patently stimulates drinking, one would expect kappa opiate agonists to stimulate water intake. Contrary to expectation, a hypodipsic response occurs only in the presence of dehydration: it is in water-deprived animals that kappa opiate agonists inhibit drinking (14). The hypodipsic and diuretic effects of kappa opiate agonists could therefore be mediated by a common mechanism responsible for a reducing of body hydration. Since naloxone completely blocks kappa opiate-mediated diuresis, it should be a suitable tool for studying interactions between the antidipsic and diuretic effects of kappa opiates. However, when administered alone, naloxone inhibits drinking (1,2). Accordingly, in our runway study, U50-induced suppression of water intake rather than being antagonized, was slightly increased by naloxone given in doses active at kappa opiate receptors (8).

A better tool for determining the role of diuresis in the kappa opiate-mediated inhibition of drinking behavior is probably the selective alpha-1 adrenergic antagonist dapiprazole (DAP) (16), which was found to inhibit, in a dose-related way, the diuretic effects of amphetamine (9,10) and U50 (9). However, because these experiments were performed in conditions of free access to water, U50 failed to influence water intake, and this failure was not affected by DAP. We have therefore evaluated whether DAP might interfere with the action of U50 on drinking behavior, in a more appropriate experimental condition, i.e., in a condition of restricted access to water. More specifically, we have studied the effects of DAP and U50 alone or in combination on

the preparatory and consummatory components of drinking in 22 h water-deprived rats negotiating water in a straight runway.

METHOD

Animals

The subjects were 8 male Sprague-Dawley rats (Morini, San Polo d'Enza, RE) initially weighing 300–500 g. They were individually housed in the laboratory in which the experiment was performed, and where temperature was maintained at 23°C with a light-dark cycle of 12 h (0700–1900).

Apparatus

The runway apparatus, already described in detail (7), consisted of a 1.8 m long alley that connected a start box to a goal box, each box measuring 35 cm long × 16 cm wide × 16 cm high. The internal surface of the starting box and alley were black, the inside of the goal box was white. A hand-operated wooden guillotine gate controlled access to the alley from the start box. Two sets of infrared photocells were inserted in the walls of the runway, 20 cm from the gate and 5 cm from the goal box entrance. The photocells allowed running time to be measured by an electronic timer. Latencies to leave the start box and to drink on reaching the goal box were measured by two hand-operated stop watches. A 200 ml water bottle was positioned on the outside of the goal box, with a 1 cm drinking spout protruding into the box, 6 cm above the floor.

Training

Rats were water restricted throughout the study by reducing daily access to water to a 20-min period. Together with water, the rats were given approximately 25 g of food. This regimen allowed the body weight of the animals to be maintained at about 85% of the previous ad lib level. When training in the runway began, water and food were given 1 h after the end of the session.

Once 85% body weight had been attained, animals were trained to obtain water in the runway, as previously described (7). During the first 5 days, for 10 min each day, the rats were given access to all the compartments of the runway, including the goal box, where water was available. During the next 10 days, each rat was submitted to a daily training session consisting of 3 trials. On each trial, the rat was placed in the starting box, and after 10 s the gate was opened. Once the rat had reached the goal box, the animal was allowed 30 s to drink. Trials were separated by 5 min. On the last 3 days of training, the rats were sham-injected before the session according to the time schedule of treatments described below. After this training period, 6 rats were selected for their consistent starting and running speeds.

Test Procedure

The test procedure differed from training in that rats were given 15 consecutive trials. In each trial, the rats were kept for 30 s in the start box before the gate was opened, and they were allowed 2 min to drink after they entered the goal box. If they failed to leave the start box within 30 s from gate opening, they were placed in the goal box by the experimenter and left there for 2 min. Sessions were conducted 6 days/week (Monday through Saturday). Tests were performed twice a week, on Tuesday and Friday.

On each test session, 15 min before the test, each rat was sequentially injected (intraperitoneally: IP) with either water, or DAP (3 or 6 mg/kg), and water, or U50 (4 or 8 mg/kg). Each animal received all 9 possible treatments according to a random sequence which was determined separately for each animal.

Data Analysis

On each trial, the following measures were taken: time to leave the start box; time to traverse the runway; interval between entering the goal box and drinking; and amount of water ingested. To normalize the data, starting times and latencies to drink were transformed into their reciprocal, while running time was transformed into running speed (m/s).

An analysis of variance (ANOVA) with subjects as blocks was performed for each parameter. The fixed variables were treatments within subjects and repeated measures within subjects and treatments (five trial blocks, each of three trials). Subsequent comparisons within logical sets of means were made using Tukey's test.

Drugs

U50, that is trans-±3,4-dichloro-N-methyl-N- [2-(1-pyrrolidinyl)-cyclohexyl)]-benzene-acetamide methane sulfonate (Upjohn Company), and DAP (Angelini, Rome) were freshly dissolved in distilled water to a final volume of 1 ml/kg.

RESULTS

As expected, running for water in basal conditions declined markedly across the test [starting speed: $F(4,20)=30.61$, $p<0.001$; running speed: $F(4,20)=34.58$, $p<0.001$; speed to drink: $F(4,20)=22.06$, $p<0.001$]. Drug treatment significantly affected starting speed, $F(8,40)=2.45$, $p=0.028$, running speed, $F(8,40)=3.66$, $p=0.053$, and speed to drink, $F(8,40)=3.13$, $p=0.007$. A significant interaction between blocks and treatments was obtained in the case of running speed, $F(32,160)=2.46$, $p<0.001$. Figure 1 only shows the data on running speed and speed to drink, since they represent better than those on starting speed the overall drug effect on running for water. Tukey's test showed that the marked reduction in running speed and speed to drink produced by U50 8 mg/kg, given alone, was responsible for the overall statistical significance of drug treatment. DAP, which was devoid of effects when given alone, prevented the inhibition produced by U50 on both running speed and speed to drink. No effects were observed when U50 4 mg/kg was administered alone or in combination with DAP (data not shown).

Water Intake

In basal conditions, rats ingested 19.4 ± 1.3 g (mean ± SEM) of water in 30 min. Water intake showed a linear decline across the test, and during the last block very little water was ingested, $F(4,20)=81.9$, $p<0.001$ (Fig. 2). The analysis of variance disclosed a significant effect of treatments, $F(8,40)=4.27$, $p<0.001$. Tukey's test showed that U50, at the dose of 8 mg/kg, reduced water intake across the test. This action was not affected by DAP treatment.

DISCUSSION

This study confirms that a selective kappa opiate agonist, such as U50, markedly inhibits the appetitive and consummatory components of drinking behavior (8). Since the animals were water-deprived for 22 h before the test, our results agree with reports of inhibition of drinking produced by kappa opiate agonists in water-deprived rats (3,14). Our study also shows that the selective alpha-1 adrenoceptor antagonist DAP prevents the

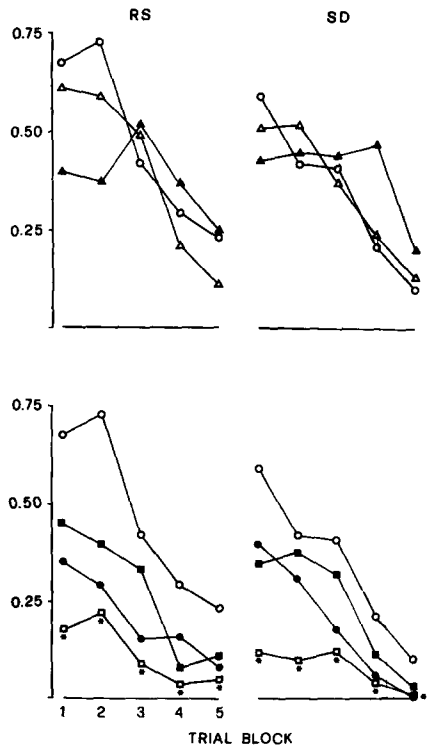

FIG. 1. Effects of DAP (△: 3 mg/kg; ▲: 6 mg/kg) given alone (upper panel) or in combination (●: 3 mg/kg; ■: 6 mg/kg) with U50 (□: 8 mg/kg; lower panel) on running speed (RS, m/s) and speed to drink (SD, s^{-1}). Each of the six rats received all the treatments shown in a random sequence, and each point shows the mean of three trials. Results obtained in control condition (○) are shown in the upper and lower panels. *$p<0.05$ vs. control condition (Tukey's test).

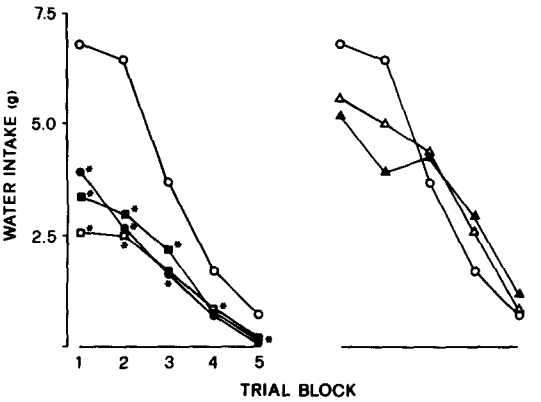

FIG. 2. Effects on water intake of DAP given either alone (right panel; △: 3 mg/kg; ▲: 6 mg/kg) or in combination (●: 3 mg/kg; ■: 6 mg/kg) with U50 8 mg/kg (left panel; □: U50 alone). Control values (○: water 1 ml/kg IP) are replicated in both panels. Each point represents the sum of the amounts of water consumed in the 3 corresponding trials. *$p<0.05$ vs. control condition (Tukey's test).

inhibitory effects of U50 on running for water, but not its effects on the amount of water ingested. When injected alone, DAP was devoid of effects on running for water and drinking. In a previous study, we have already ruled out the possibility that the slowing of running for water is due to the sedative properties of U50 (8). This is confirmed by the antagonist action of DAP on U50, since another selective alpha-1 antagonist, prazosin, has been found to enhance U50-mediated sedation in mice (5). Thus the influence that DAP exerted on U50 effects may be specific for drinking behavior. This is in line with the hypothesis that the antidipsic action of kappa opioids may be mediated by alpha adrenergic mechanisms (6). Our study suggests that these mechanisms involve alpha-1 adrenoceptors, and that their role is restricted to the preparatory aspects of drinking.

Why DAP prevented the suppressant effects of U50 on running for water without affecting the inhibition of the actual water intake across the test is not clear. Considering that both U50 and naloxone suppressed water intake, but only U50 slowed running for water, we previously suggested at least a partial nonoverlap of the mechanisms underlying the preparatory and consummatory components of drinking (8). This speculation is substantiated by the present finding that blocking alpha-1 adrenoceptors with DAP makes the response to U50 similar to the effect we have previously observed with naloxone administration: water intake is suppressed, but running for water is not (8). Thus, incidentally, this study narrows down the investigation on mechanisms responsible for the puzzling overlap in the response to a kappa opioid agonist and to a nonselective opioid antagonist to the consummatory component of drinking.

The role of alpha-1 adrenoceptors in the diuresis and inhibition of searching for water produced by U50, suggests that the two effects are integrated in a common mechanism of water dissipation activated by kappa opioids. In our experimental conditions, however, drinking behavior was measured during the 45 min after U50 administration, when the diuretic effect was presumably not fully expressed. Whether DAP still antagonizes the inhibition of running for water in later phases of the time course of U50 effects is unknown. Nevertheless, in the natural setting, early inhibition of searching for water could help to prevent attempts to compensate for dehydration produced by diuresis. Although highly speculative, this suggestion merits further study.

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