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Inhibition of Drinking by Naltrexone in the Rat: Interaction With the Dopamine D-1 Antagonist SCH 23390 and the D-2 Antagonist Sulpiride

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UKAI, M., S. NAKAYAMA AND T. KAMEYAMA. *Inhibition of drinking by naltrexone in the rat: Interaction with the dopamine D-1 antagonist SCH 23390 and the D-2 antagonist sulpiride*. PHARMACOL BIOCHEM BEHAV **32**(3) 651–655, 1989.—The involvement of dopamine receptors in water intake was investigated in the rat deprived of water for 24 hr. A 0.03 mg/kg dose of SCH 23390 markedly enhanced naltrexone (0.1 and 10.0 mg/kg)-induced hypodipsia, whilst the drug alone significantly decreased water intake at doses of 0.01 to 3.0 mg/kg, accompanied by marked motor dysfunction. Sulpiride (20.0 and 40.0 mg/kg) did not markedly affect water intake and naltrexone-induced hypodipsia. Consistent with previous results, apomorphine (0.3 mg/kg) alone was without marked effects, while it produced a marked potentiation of naltrexone (1.0 and 10.0 mg/kg)-induced hypodipsia. SCH 23390 (0.003 mg/kg) and sulpiride (40.0 mg/kg) completely antagonized the enhancing effects of apomorphine on naltrexone-induced hypodipsia. Similar effects were also seen in the latency to begin drinking. In contrast to the effects on naltrexone-induced hypodipsia, it appears that both dopamine D-1 and D-2 receptors play a key role in the effects of apomorphine on naltrexone-induced hypodipsia in the rat.

SCH 23390 Sulpiride Naltrexone Apomorphine Water intake

IT is well known that opioid systems are closely associated with ingestive behaviors (14,21). Particularly, the effects of opioid antagonists on drinking are consistent in contrast to those of opioid agonists (5, 8, 24, 26, 28). Naloxone and naltrexone inhibit deprivation- and hypertonic saline-induced water intake in rodents (1–4). In addition, the injection of naltrexone methobromide into the lateral ventricle has been reported to attenuate drinking (3,26). A recent study has shown that the administration of naltrexone methobromide into the paraventricular and supraoptic hypothalamic nuclei produces a significant decrease in deprivation (24 hr)-induced water intake (26). It has been reported, however, that even higher doses of opioid antagonists do not completely abolish water intake in the water-deprived rat (1,26), thus indicating that other neuronal systems besides opioids should be involved in drinking. There is a possibility that acetylcholine, γ-aminobutyric acid (GABA), norepinephrine, dopamine, and neuropeptide Y participate in ingestive behaviors (6, 9, 11, 12, 22, 29). Particularly, Poat *et al.* (18) have demonstrated that the intracerebral injection of dopamine stimulates drinking in the rat, whereas dopamine receptor antagonists decrease it. At present, dopamine receptors have at least been divided into two types such as D-1 and D-2 based upon neurochemical and pharmacological profiles (23). From these points, it would particularly be of interest to clarify the function of dopamine receptor subtypes in drinking. Since there have been numerous reports indicating the close interaction of enkephalins with dopaminergic neurons in the central nervous system (10, 16, 19), the present study was designed to unravel the effects of two dopamine antagonists such as the D-1 selective SCH 23390 and the D-2 selective sulpiride on naltrexone-induced hypodipsia. Subsequently, the effects of the selective antagonists on the potentiating effects of apomorphine on naltrexone-induced hypodipsia were tested, since we have very recently reported the potentiating effects of apomorphine without causing motor deficit (27).

METHOD

Animals

Male Wistar rats (Shizuoka, Japan) weighing between 250 and 350 g were used. The animals were randomly assigned to groups consisting of 8 to 10 rats per group. Between experiments, the rats were housed 3 or 4 per cage and maintained on a 12 hr light-dark schedule (lights on at 0800) with food and water freely available.

Procedure

Rats were adapted to a water deprivation schedule. At least 3 familiarization trials with saline injected systemically preceded actual drug testing. All subjects were deprived of water in their home cage for 24 hr before the start of each test session. Food was

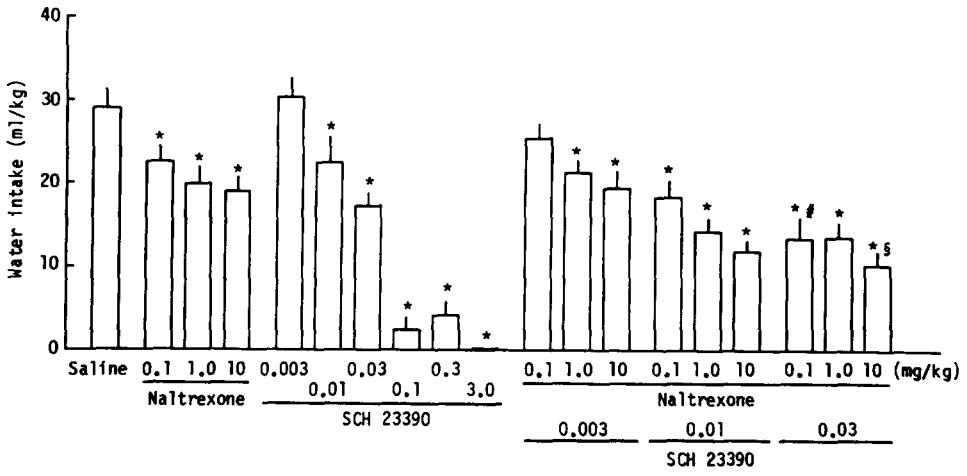

FIG. 1. Effects of naltrexone, SCH 23390 and their combinations on water intake in the rat deprived of water for 24 hr. $\star p<0.05$ vs. saline, $\#p<0.05$ vs. 0.1 mg/kg of naltrexone, $\S p<0.05$ vs. 10.0 mg/kg of naltrexone.

available at all times except during subsequent test periods. In each test session, animals were put into individual cages and allowed access for 30 min to water from 25 ml graduated cylinders fitted with drinking spouts. The latency to begin drinking was measured in addition to the volume of water consumed. On the test days, rats were treated with one of the drugs. Each rat received all treatments with the drug in a random sequence that included saline as a control. Although motor behavior was not analysed and quantified during drinking test, it was carefully checked by a trained observer. Tests were performed once or twice weekly between 0930 and 1400.

Data Analysis

Each value was converted to volume of water (ml) consumed per kg body weight for drinking tests. The data obtained were analysed by two-factor analysis of variance (ANOVA) for randomized blocks. Post hoc analysis was conducted by the Newman-Keuls method (30). A p value of less than 0.05 was taken as the level of statistical significance.

Drugs

Drugs used were apomorphine hydrochloride and racemic sulpiride (Sigma), naltrexone hydrochloride (DuPont), and SCH 23390 maleate (Schering). Apomorphine (IP), naltrexone (SC), SCH 23390 (IP) and sulpiride (IP) were administered 15, 30, 60 and 60 min, respectively, before the test for drinking. Doses are expressed in terms of the free base of the drugs.

RESULTS

Effects of SCH 23390

ANOVA showed a significant influence on water intake, $F(18,126)=22.4$, $p<0.005$ (Fig. 1). Naltrexone (0.1, 1.0 and 10.0 mg/kg) decreased dose-dependently water intake in the rat deprived of water for 24 hr (Fig. 1). Although a 0.003 mg/kg dose of SCH 23390 failed to influence water intake, higher doses (0.01–3.0 mg/kg) significantly reduced water intake, accompanied by marked motor deficit. A dose of 0.003 or 0.01 mg/kg of the drug did not significantly enhance naltrexone-induced hypodipsia, whilst a 0.03 mg/kg dose which alone produced a marked decrease in water intake augmented naltrexone (0.1 and 10.0 mg/kg)-induced hypodipsia (Fig. 1). Naltrexone, SCH 23390 or their combinations did not affect the latency to start drinking in the rat deprived of water for 24 hr (data not shown).

Effects of Sulpiride

ANOVA revealed a significant effect on water intake, $F(11,88)=3.4$, $p<0.005$ (Fig. 2). A dose of 20.0 or 40.0 mg/kg of sulpiride did not influence deprivational water intake, and further a 40 mg/kg dose failed to produce a marked potentiation of naltrexone (0.1, 1.0 and 10.0 mg/kg)-induced hypodipsia (Fig. 2). Sulpiride, naltrexone or their combinations had no significant effects on the latency to begin drinking in the rat deprived of water for 24 hr (data not shown).

Effects of SCH 23390 and Sulpiride on the Effects of Apomorphine

ANOVA displayed a significant effect on water intake, $F(11,99)=38.9$, $p<0.005$ (Fig. 3). Apomorphine (0.3 mg/kg) markedly potentiated naltrexone-induced hypodipsia similar to the effects reported previously (Fig. 3). When combined with SCH 23390 (0.003 mg/kg) or sulpiride (40.0 mg/kg), although they themselves had no marked effects on either deprivational water intake or naltrexone-induced hypodipsia, the effects of apomorphine on naltrexone-induced hypodipsia were almost completely reversed (Fig. 3). ANOVA also showed a significant influence on latency to begin drinking, $F(11,99)=12.0$, $p<0.005$ (Fig. 4). The same trend was seen in the case of the latency (Fig. 4).

DISCUSSION

Naltrexone (0.1–10.0 mg/kg) reduced dose-dependently deprivation (24 hr)-induced water intake similar to previous results (1–4). The effects were significant but rather incomplete, again indicating the involvement of other neuronal systems besides opioids in drinking. Although a 0.003 mg/kg dose of SCH 23390 failed to influence deprivation (24 hr)-induced water intake, higher doses (0.01–3.0 mg/kg) significantly reduced the intake. Especially, a 3.0 mg/kg dose almost completely abolished it. Similar results have been obtained by Gilbert and Cooper (7). SCH 23390 at a dose of 0.03 mg/kg potentiated naltrexone (0.1 and 10.0

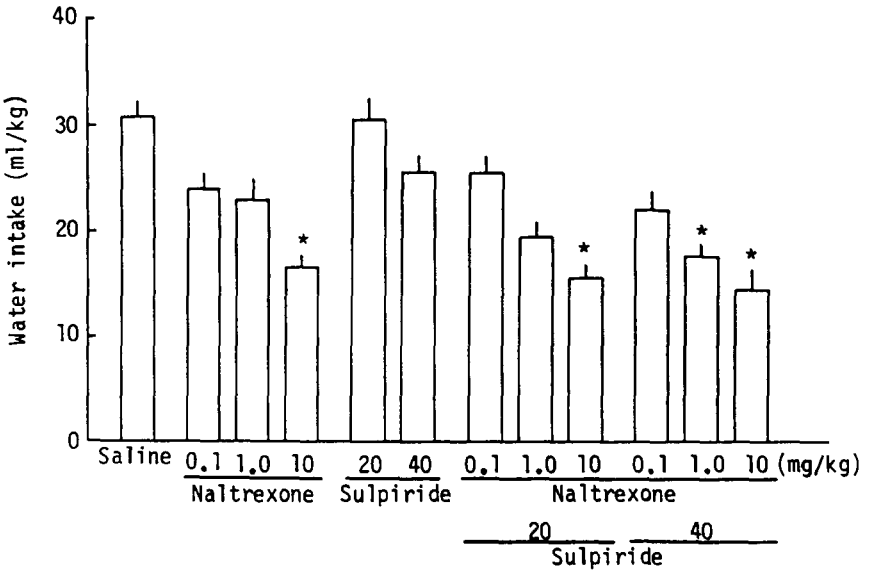

FIG. 2. Effects of naltrexone, sulpiride and their combinations on water intake in the rat deprived of water for 24 hr. $\star p<0.05$ vs. saline.

mg/kg)-induced hypodipsia, but the dose itself significantly inhibited deprivation (24 hr)-induced water intake. Since more than 0.025 mg/kg doses of SCH 23390 are likely to evoke catalepsy (13), the potentiating effects may be due to motor dysfunction. Additionally, the dopamine D-2 receptor antagonist sulpiride (20.0 and 40.0 mg/kg) did not markedly augment naltrexone-induced hypodipsia, also suggesting that the selective antagonists per se do not play an important role in naltrexone-induced hypodipsia in contrast to the effects of haloperidol on dynorphin-(1–13)-induced polyphagia (15).

The results additionally show that apomorphine (0.3 mg/kg) markedly potentiated naltrexone-induced hypodipsia consistent with previous evidence. Since a 0.3 mg/kg dose is considered to be a "postsynaptic" dose (20), the potentiation by apomorphine may be mediated via postsynaptic dopamine receptors (27). Therefore, the increase in dopaminergic neuronal activity would result in

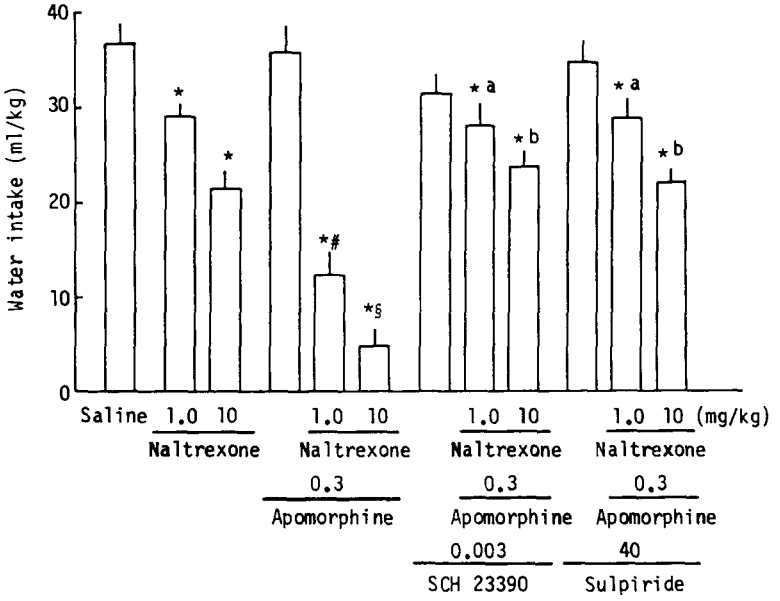

FIG. 3. Effects of SCH 23390 and sulpiride on the potentiating effects of apomorphine on naltrexone-induced hypodipsia in the rat deprived of water for 24 hr. $\star p<0.05$ vs. saline, $\#p<0.05$ vs. 1.0 mg/kg of naltrexone, $\S p<0.05$ vs. 10.0 mg/kg of naltrexone, $^{a}p<0.05$ vs. 1.0 mg/kg of naltrexone plus 0.3 mg/kg of apomorphine, $^{b}p<0.05$ vs. 10.0 mg/kg of naltrexone plus 0.3 mg/kg of apomorphine.

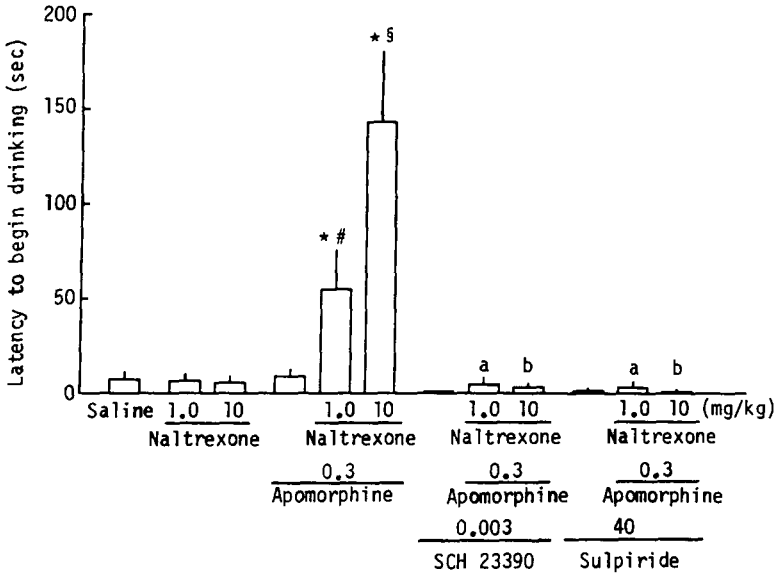

FIG. 4. Effects of SCH 23390 and sulpiride on the prolonging effects of apomorphine on the latency to begin drinking in the rat deprived of water for 24 hr. ★$p<0.05$ vs. saline, #$p<0.05$ vs. 1.0 mg/kg of naltrexone, §$p<0.05$ vs. 10.0 mg/kg of naltrexone, $^{a}p<0.05$ vs. 1.0 mg/kg of naltrexone plus 0.3 mg/kg of apomorphine, $^{b}p<0.05$ vs. 10.0 mg/kg of naltrexone plus 0.3 mg/kg of apomorphine.

enhancing naltrexone-induced hypodipsia.

Dopamine antagonists seem to be only active for potentiating effects of apomorphine on naltrexone-induced hypodipsia. SCH 23390 (0.003 mg/kg) and sulpiride (40.0 mg/kg), which themselves did not affect deprivation (24 hr)-induced water intake, almost completely reversed the effects of apomorphine. Similar outcome was observed in the latency to begin drinking. It is thus likely that both dopamine D-1 and D-2 receptors have a key function in the effects of apomorphine. On the contrary, SCH 23390 antagonized the actions of apomorphine on naltrexone-suppressed drinking at doses that were 4 orders of magnitude lower than those required of sulpiride. This would suggest that D-1 receptors are more closely involved in the potentiating actions of apomorphine, even though complete pharmacological data are not available. The evidence also implies that dopamine interacts with enkephalins at postsynaptic sites, because it has been reported that autoreceptors located on dendrites and cell bodies of dopamine-containing neurons are not directly acted upon by SCH 23390 (17). Ukai and Holtzman (25,26) have demonstrated that, when microinjected, DAGO [D-Ala^2-NMePhe^4-Gly(ol)-enkephalin] or naltrexone methobromide the paraventricular hypothalamic nucleus is a most sensitive site in drinking behaviors, further suggesting that potential central mechanisms between dopamine and enkephalins exist in the above site.

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