Attenuation by glucose of the hyperactivity induced in mice by combined tripeleennamine and morphine

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The present study examined the effects of glucose on the hyperactivity induced by morphine (10 mg/kg), given alone or combined with the histamine H₁-receptor antagonist tripeleennamine (2.5 or 5 mg/kg), in mice of the CD-1 strain. In the first experiment, glucose (50, 100, 250, or 500 mg/kg) had no significant effect on spontaneous locomotor activity, but at the dose of 100 mg/kg significantly reduced morphine-induced hyperactivity. In the second experiment, the administration of glucose (50 or 100 mg/kg) was also able to attenuate the locomotor stimulation induced by combined tripeleennamine and morphine. The results are consistent with the hypothesis that glucose may inhibit opiate functions and indicate that glucose may exert an inhibitory action even on the central action of combinations of opiate and antihistaminic agents, drug combinations able to produce additive euphoric effects in humans.

Several animal studies have reported interferences by glucose of the effects exerted by various centrally acting drugs in learning and memory (Kopf & Baratti, 1994, 1996; Stone, Croul, & Gold, 1988; Stone, Walser, Gold, & Gold, 1991) as they have in other behavioral tests (Stone, Cottrill, Walker, & Gold, 1988; Stone, Rudd, & Gold, 1990). Considerable evidence indicates that glucose interferes with cholinergic mechanisms and with the effects of cholinergic agents (Kopf & Baratti, 1994, 1996; Pavone, Capone, Battaglia, & Sansone, 1998; Stone, Cottrill, & Gold, 1987; Stone, Cottrill, et al., 1988; Stone, Croul, & Gold, 1988), but interferences by glucose of the opiate mechanisms have also been reported (Stone et al., 1991). In particular, glucose attenuates morphine-induced analgesia (Lux, Brase, & Dewey, 1988; Simon & Dewey, 1981) and prevents behavioral effects of the opiate, such as locomotor stimulation (Stone et al., 1990) and impairments of inhibitory avoidance and spontaneous alternation (Ragozzino, Parker, & Gold, 1992; Stone et al., 1991).

Previous studies have shown that morphine-induced hyperactivity might be enhanced by some antihistaminic drugs, such as tripeleennamine and other histamine H₁-receptor blockers (Leza, Lizasoain, & Lorenzo, 1991; Sansone, Castellano, & D'Amato, 1986; Sansone, D'Udine, Renzi, & Vetulani, 1987). In the present study, we investigated whether glucose, at doses that reduced the hyperactivity induced by morphine alone, would be able to also attenuate the locomotor stimulation produced by the opiate combined with tripeleennamine. Even if less effective than other antihistaminics in enhancing morphine-induced hyperactivity (Sansone et al., 1986; Sansone et al.,

1987), tripeleennamine was chosen for the present study because experimental research on antihistaminic-opiate combinations had been prompted by reports indicating a considerable abuse by heroin addicts of the narcotic agonist-antagonist pentazocine combined with tripeleennamine ("Ts and blues"), in certain areas of the United States (Lahmeyer & Steingold, 1980; Showalter, 1980). Combination of the two drugs produced additive euphoric effects in human subjects (Jasinski, Johnson, & Henningfield, 1984).

In the present study, the first experiment confirmed the ability of glucose to reduce morphine-induced hyperactivity in our experimental conditions too. The second experiment demonstrated attenuation by glucose of the locomotor stimulation induced by combined tripeleennamine and morphine.

METHOD

Subjects

The subjects were naive male mice, 8–9 weeks old, of the randomly bred CD-1 strain (Charles River, Calco-Como, Italy). Upon their arrival in the laboratory (at least 1 week before the experiment), the mice were housed in standard transparent plastic cages (4 per cage) under standard animal-room conditions: free access to food and water, 12:12-h light:dark cycle (lights on at 7:00 a.m.), ambient temperature of 23°C. The experiments were carried out between 9:00 a.m. and 1:00 p.m.

Care and handling of the animals were in accordance with NIH ethical regulations. The experimental protocol was approved by the Italian Ministry of Health on November 27, 1995 (Decree 285/95-B).

Apparatus

Locomotor activity was measured in a computer-controlled apparatus consisting of eight toggle-floor boxes, each divided into two 20 × 10 cm compartments connected by a 3 × 3 cm opening. For each mouse the number of crossings from one compartment to the other was recorded by means of a microswitch connected to the tilt-

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ing floor of the box. The apparatus was located in a sound-insulated cubicle.

Drugs

Glucose (D-glucose, anhydrous, Sigma), tripeleannamine hydrochloride (Sigma), and morphine hydrochloride (Ghieu), dissolved in distilled water, were injected intraperitoneally in a volume of 10 ml/kg. Control injections (dose 0 of the drugs) consisted of the administration of saline solution (0.9% NaCl).

Procedure

In the first experiment, the animals were injected with glucose (0, 50, 100, 250, or 500 mg/kg) and, 15 min later, with morphine hydrochloride (0 or 10 mg/kg). A 30-min activity test started 15 min after the second injection. Each experimental group included 16 subjects.

In the second experiment, glucose (0, 50, or 100 mg/kg) and tripeleannamine hydrochloride (0, 2.5, or 5 mg/kg) were given as a mixed solution, in a single injection, 30 min before testing. Fifteen minutes after the first treatment, the mice received a second injection with morphine hydrochloride (0 or 10 mg/kg). Groups receiving saline as the second injection (dose 0 of morphine) consisted of 16 subjects; groups receiving morphine consisted of 24 mice.

Statistical Analysis

The results were analyzed by nonparametric statistical methods. Median number of crossings and semi-interquartile range (Q1–Q3) were calculated for each experimental group. Significance of the differences among the groups receiving the same dose of glucose was evaluated by means of the Kruskal-Wallis one-way analysis of variance (ANOVA); individual between-group comparisons were performed using the Mann-Whitney *U* test.

RESULTS

The results of the first experiment, concerning the effects of glucose and morphine, given alone or combined, are reported in Figure 1, as median number of crossings recorded during 30 min, for each experimental group. A Kruskal-Wallis ANOVA indicated a significant difference among the groups [$H(9) = 41.67$, $p < .001$]. Individual between-group comparisons (Mann-Whitney *U* test) revealed no significant effect ($ps > .05$) of the four tested doses of glucose given alone, while morphine alone (10 mg/kg) significantly ($p < .01$) increased the number of activity crossings. Glucose, even if ineffective alone, reduced morphine-induced hyperactivity significantly at the dose of 100 mg/kg. In particular, mice receiving morphine following after 100-mg/kg glucose exhibited a number of activity crossings significantly lower than that of mice treated with morphine alone ($p < .05$) and not significantly different from that of mice treated with glucose alone ($p > .05$).

Figure 2 shows the effects on locomotor activity of combined tripeleannamine and morphine in the absence of glucose treatment (GLU 0) and in mice receiving glucose simultaneously with tripeleannamine (GLU 50 and GLU 100). Kruskal-Wallis ANOVAs indicated a significant difference among the groups for each dose of glucose [$H(5) = 52.93$, $p < .001$; 29.26 , $p < .001$; and 12.36 , $p < .05$, for glucose 0, 50, and 100, respectively]. Individual between-group comparisons indicated no significant effect of tripeleannamine, administered without mor-

Figure 1. Locomotor activity, measured during 30 min, in mice receiving saline solution or morphine hydrochloride (10 mg/kg), 15 min after the administration of glucose. The activity test started 15 min after the second injection. Columns represent median crossings; vertical lines indicate semi-interquartile ranges. Significances by the Mann-Whitney *U* test: * $p < .05$, ** $p < .01$, morphine versus the corresponding saline group, and * $p < .05$, versus morphine alone (dose 0 of glucose).

phine, in the absence of glucose as in glucose-treated mice ($ps > .05$). Morphine alone significantly ($p < .01$) increased locomotor activity in mice that did not receive glucose. In the absence of glucose, combinations of tripeleannamine and morphine increased locomotor activity more than did morphine alone, but such enhancement was not statistically significant ($ps > .05$). Glucose, at the dose of 50 mg/kg, did not change the effect of morphine alone ($p > .05$) but did prevent the locomotor stimulation induced by morphine combined with 5-mg/kg tripeleannamine ($p < .05$). At the dose of 100 mg/kg, glucose reduced the hyperactivity induced either by morphine alone ($p < .01$) or by combined tripeleannamine and morphine ($ps < .05$).

Enhancement of morphine-induced hyperactivity by tripeleannamine and the related attenuating action of glucose were more evident, and statistically significant, when the data analysis was applied to the first 15 min of the 30-min activity test. Median crossings (Mdn; interquartile ranges in parentheses) were calculated for all morphine-treated groups of mice: morphine alone or combined with 2.5- and 5-mg/kg tripeleannamine, Mdn = 101.0 (71–122), 127.0 (86–157), and 122.5 (90–137), respectively, in the absence of glucose; Mdn = 89.5 (80–123), 105.5 (84–130), and 80.0 (55–145), in the presence of 50-mg/kg glucose; Mdn = 69.0 (48–96), 101.5

Figure 2. Locomotor activity, measured during 30 min, in mice receiving saline solution or morphine hydrochloride (10 mg/kg) 15 min after the administration, in a single injection, of glucose (GLU: 0, 50, or 100 mg/kg) and tripeleennamine hydrochloride. The activity test started 15 min after the second injection. Columns represent median crossings; vertical lines indicate semi-interquartile ranges. Significances by the Mann-Whitney *U* test: * $p < .05$, ** $p < .01$, morphine versus the corresponding saline group; * $p < .05$, ** $p < .01$, versus GLU 0, at corresponding doses of tripeleennamine and morphine.

(56–121), and 91.0 (63–131) in the presence of 100-mg/kg glucose. The Mann-Whitney *U* test showed that the hyperactivity induced by morphine, in the absence of glucose, was significantly enhanced by both doses of tripeleennamine ($p < .05$). The effects of glucose were similar to those described for the whole 30-min activity test. At the dose of 50 mg/kg, glucose significantly reduced the locomotor stimulation induced by morphine combined with 5 mg/kg tripeleennamine ($p < .05$). At the dose of 100 mg/kg, glucose significantly attenuated the hyperactivity induced either by morphine alone or by combined tripeleennamine and morphine ($p < .05$).

DISCUSSION

In agreement with previous findings (Stone et al., 1990), peripheral administration of glucose in the present study

did not affect spontaneous locomotor activity in mice but did reduce morphine-induced hyperactivity. Glucose was also able to attenuate the locomotor stimulation produced by morphine combined with the histamine H_1 -receptor blocker tripeleennamine. Combinations of tripeleennamine and morphine, as previously reported (Sansone et al., 1986; Sansone et al., 1987), increased locomotor activity more than did morphine alone, the effect being statistically significant during the first half of the 30-min activity test.

The neurochemical mechanisms involved in the central action of glucose are not yet clear, but consistent evidence suggests that glucose administration may augment central cholinergic functions and impair opiate functions (Stone et al., 1991). A cholinergic activation by glucose has been demonstrated by behavioral studies showing that glucose administration attenuates the hyperactivity pro-

duced by the muscarinic antagonist scopolamine (Stone et al., 1987) and enhances tremors induced by physostigmine, a cholinomimetic agent acting through acetylcholinesterase inhibition (Stone, Cottrill, et al., 1988). An enhancement of the central cholinergic function seems to play an important role even in the improving effects exerted by glucose in learning and memory tasks (Kopf & Baratti, 1994, 1996; Pavone et al., 1998; Stone, Croul, & Gold, 1988). The cholinomimetic action of glucose might be due to a facilitation of synthesis and/or release of acetylcholine (Kopf & Baratti, 1996; Ragozzino, Unick, & Gold, 1996). On the other hand, an inhibitory action of glucose on central opiate functions has been assumed on the basis of experimental evidence indicating that glucose may antagonize the antinociceptive action of morphine (Lux et al., 1988; Simon & Dewey, 1981) and may reverse morphine-induced impairments of inhibitory avoidance and spontaneous alternation performance (Ragozzino et al., 1992; Stone et al., 1991). Reduction by hyperglycemia of morphine effects might be due to reduced binding of the drug to opiate receptors (Shook, Kachur, Brase, & Dewey, 1986), but such a hypothesis, supported by a previous neurochemical investigation (Brase, Han, & Dewey, 1987), was not confirmed by further experimental findings (Khawaja & Green, 1992).

Both the inhibition of opiate systems and the enhancement of cholinergic activity may contribute to producing the attenuating action of glucose on morphine-induced hyperactivity (Stone et al., 1990). Since cholinergic activation may be due, at least in part, to opiate inhibition, Stone et al. (1990) suggested that the attenuation of morphine-induced hyperactivity by glucose might be ascribed to a glucose-cholinergic-opiate interaction. It is likely that the same neurochemical mechanisms are involved in the attenuation by glucose of the locomotor stimulation induced by tripeleminamine-morphine combinations, but the involvement of other neurotransmitter systems cannot be excluded. In fact, besides their specific H_1 -receptor-blocking activity, antihistaminics interact with various neurotransmitter systems (Douglas, 1985), so that interactions with the neurotransmitters mediating the behavioral effects of morphine may occur downstream of the opiate receptors (Sansone et al., 1987). However, dopamine, noradrenaline, and particularly serotonin are the neurotransmitters that seem to be mainly involved in the central action of histamine H_1 receptor blockers and in their interaction with opiates (San-Martin-Clark, Cuellar, Leza, Lizasoain, & Lorenzo, 1996; Suzuki, Mori, Tsuji, Misawa, & Onodera, 1997), but at present no information is available on the effects of peripheral administration of glucose on central monoaminergic systems.

In conclusion, the present study demonstrates that peripheral administration of glucose attenuates the locomotor stimulation induced in mice by combined tripeleminamine and morphine. Further research should investigate whether glucose is also able to attenuate the effects of combined antihistaminics and opiates in other behavioral tests. Besides morphine-induced hyperactivity, in

fact, other effects of the opiates—that is, the antinociceptive action—may be reduced by glucose (Lux et al., 1988; Simon & Dewey, 1981) and enhanced by antihistaminic drugs (Bluhm, Evans, & Zsigmond, 1983; Sun, Hui, & Hanig, 1985; Tagashira, Kachur, Carter, & Dewey, 1984; Yeh, 1986). But it might certainly be of great interest to ascertain the action of glucose on the euphoric effects produced by antihistaminic-opiate combinations in humans.

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