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Voluntary Morphine Ingestion, Morphine Dependence, and Recovery from Withdrawal Signs[1]

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KHAVARI, K. A., T. C. PETERS, P. L. BAITY AND A. S. WILSON. *Voluntary morphine ingestion, morphine dependence, and recovery from withdrawal signs.* PHARMAC. BIOCHEM. BEHAV. 3(6) 1093–1096, 1975. – We are reporting on conditions (without forced drinking or premedications) where rats voluntarily drink high quantities of sucrose-morphine solutions in preference to water. The volume ingested is inversely related to the morphine concentration in the liquid. The morphine antagonist, nalorphine, produced a clear set of opiate withdrawal signs in these (voluntarily) morphine-drinking rats. The severity of withdrawal signs was a function of the amount of ingested morphine. The present finding is the first report to show that rats ingest high quantities of sucrose-morphine without premedication or forced hydration procedures.

Morphine Nalorphine Voluntary ingestion Withdrawal signs Rats

IN recent years, a number of procedures and techniques have been developed for investigation of opiate self-administration in laboratory animals. The intravenous [1,8] and the oral [3, 4, 5, 7] methods have been employed effectively. Nichols [5] and his colleagues generally employed a premedication procedure prior to a two-bottle choice test, i.e., the animals were injected with morphine for a number of days and then were given an opportunity to drink either water or water laced with morphine. According to Nichols' escape model, animals premedicated with morphine drink a morphine solution (which is refused by nonmedicated animals) in order to avoid the stress produced by withholding morphine injections. Nichols and his associates, employing the premedication procedure, have been able to increase rats' preference for orally consumed morphine in a two-bottle choice situation. Stolerman and Kumar [7] have employed a hydration procedure which does not require premedication for development of morphine preference in rats. In their procedure, 17 hr water-deprived rats were given a 7 hr choice test between water and a morphine solution (0.5 mg/ml) on Day 1. On Days 2 and 3 the rats were provided with only the morphine solution during the 7 hr drinking period. This cyclical procedure was continued, generally for some 40 days. The above investigators observed a gradual occurrence of increased morphine solution ingestion. More recently, Khavari and Risner [3] provided rats with a single source of liquid (0.5 mg morphine HCl/ml of 10 percent sucrose) and observed that rats drink high quantities of the fluid (approximately 120 ml/day/rat) for many days.

All procedures described above have been effective in bringing about opiate self-administration by animals. However, they all involve injection, premedication, or forced hydration methods. Laboratory animals, when not pretreated, refuse fluids which contain any significant amount of morphine in two-bottle choice tests.

The present study was designed to examine the following points. First. It is common knowledge that rats consume high quantities of sucrose solutions in preference to water [2]. It is also suspected that the bitter taste of morphine is an important factor in rats' refusal to drink appreciable quantities of morphine-water when the animals

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are neither pretreated nor forced through hydration procedures [4]. Therefore, we wished to examine the effectiveness of sucrose in overriding the aversive taste of morphine. Second. We wished to examine morphine self-administration under a free choice condition involving neither pretreatment nor forced procedures. Third. We wished to determine the morphine concentration which would constitute the optimal dose for voluntary drinking of sucrose morphine solution. Fourth. We set out to examine the parameters of morphine dependence, withdrawal signs, and the course of recovery from withdrawal precipitated by administration of the opiate antagonist, nalorphine.

METHOD

Animals

The animals were 60 male Sprague-Dawley rats weighing 400–460 g at the start of the experiment. Each rat was individually housed in a standard laboratory cage in a constantly lighted environment with free access to milled Wayne rat food. In addition, each rat was provided with 2 bottles of tap water. The animals were assigned randomly to 6 equal groups. For 7 days body weight, food intake, and drinking from the 2 bottles were recorded daily. We found that all animals ingested water from both bottles, with a slight bias for the bottle closer to the food container. Therefore, bottle positions were rotated daily during the next phase of the experiment.

Procedure

After this initial 7 day observation the choice phase was initiated. Each group of 10 rats was randomly assigned to a particular sucrose-morphine solution. One of the 2 bottles, for all groups, contained water while the other bottle contained a sucrose-morphine solution. Morphine contents were 0.125, 0.25, 0.5, 1.0, 1.5 and 2.0 mg of morphine HCl in every milliliter of the 10 percent sucrose. All 6 groups were maintained on this 2 bottle choice phase for 17 days. Measures of body weight, food intake, and sucrose-morphine intake were taken daily. At the end of Day 17 of the choice phase, the first 3 groups (morphine groups 0.125, 0.25, and 0.5 mg/ml) were injected IP with 4 mg/kg of the morphine antagonist nalorphine HCl. In addition, they were no longer provided with their respective sucrose-morphine solutions. During this withdrawal phase, which lasted for 9 days, each rat was given ad lib access to 1 bottle of water, 1 bottle of 10 percent sucrose (the original morphine vehicle for all groups), and milled food. Again, measures of body weight, food, water and sucrose intakes were taken daily. During the 17 day free choice phase none of the 3 higher morphine concentration (1.0, 1.5 and 2.0 mg/ml) groups ingested any appreciable quantities of their respective sucrose-morphine solutions. Therefore, the above 3 groups were dropped from the study. Six rats constituted the 0.5 morphine concentration groups and 10 animals in each of the other 2 lower groups.

RESULTS AND DISCUSSION

Figure 1 shows the mean daily fluid ingestion for the 3 groups during 7 days of water only and 17 days of two-bottle choice. Mean daily water intake during the 7 days of prechoice baseline was about 50 ml for all animals. During Day 1 of the choice phase, only the lowest

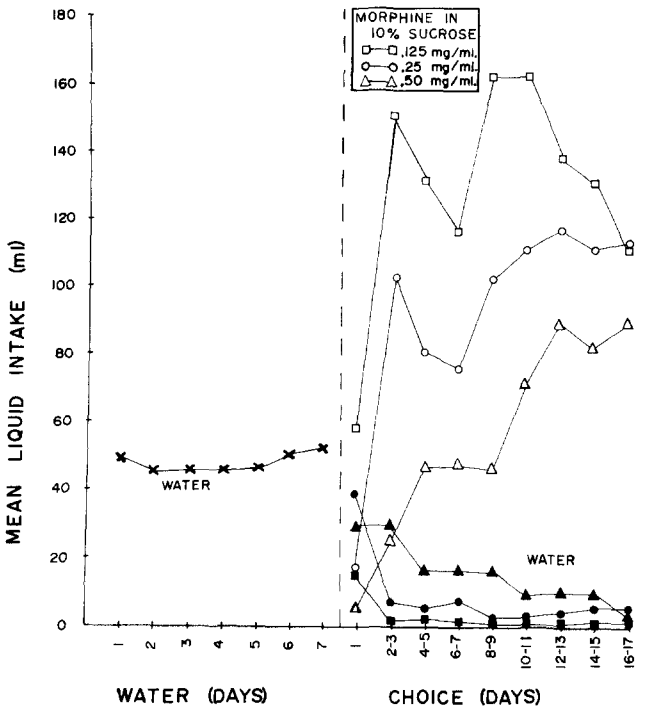

FIG. 1. Mean daily water intake of rats from 2 adjacent water bottles during baseline (left). Mean daily intake of sucrose-morphine and water from 2 adjacent bottles during 17 days of free choice for 3 groups of rats (right). It is clear that all 3 groups rapidly began to ignore the water and consume high daily quantities of their respective sucrose morphine.

sucrose-morphine concentration group showed a clear preference for the sucrose-morphine over water. By Day 5 of this phase all 3 groups were ingesting significantly more sucrose-morphine than water. There was an overall increase in sucrose-morphine intake for the 3 groups during the 17 days of choice phase, $F(2,23) = 35.5$, $p<0.01$. In contrast, water intake of the 3 groups showed a reliable decline over the same 17 day period, $F(2,23) = 9.4$, $p<0.01$. The amount of morphine ingested by the 3 groups also increased during the choice period, $F(2,23) = 13.5$, $p<0.01$. The amount of morphine ingested was a function of the drug concentration in the sucrose vehicle (Fig. 2). Specifically, during the last 6 days of the choice phase the mean daily intake of morphine was approximately 45 mg for the 0.5 concentration group, 28 mg for the 0.25 concentration group, and only 14 mg for the 0.125 concentration group. Therefore, it is apparent that the rats began to drink the morphine-adulterated sucrose solution because of the highly reinforcing 10 percent sucrose. The amount of morphine in the standard 10 percent sucrose clearly affected their intake. This point is obvious when ingestion data from the 3 higher morphine concentration groups are considered. Morphine concentration of 1 mg/ml and higher apparently override the reinforcing potency of the 10 percent sucrose, for none of the animals in the 3 higher morphine concentration groups ingest any appreciable quantities of these solutions.

The above data reveal, for the first time, conditions under which rats would voluntarily ingest high quantities of morphine in a sucrose vehicle without any premedication or

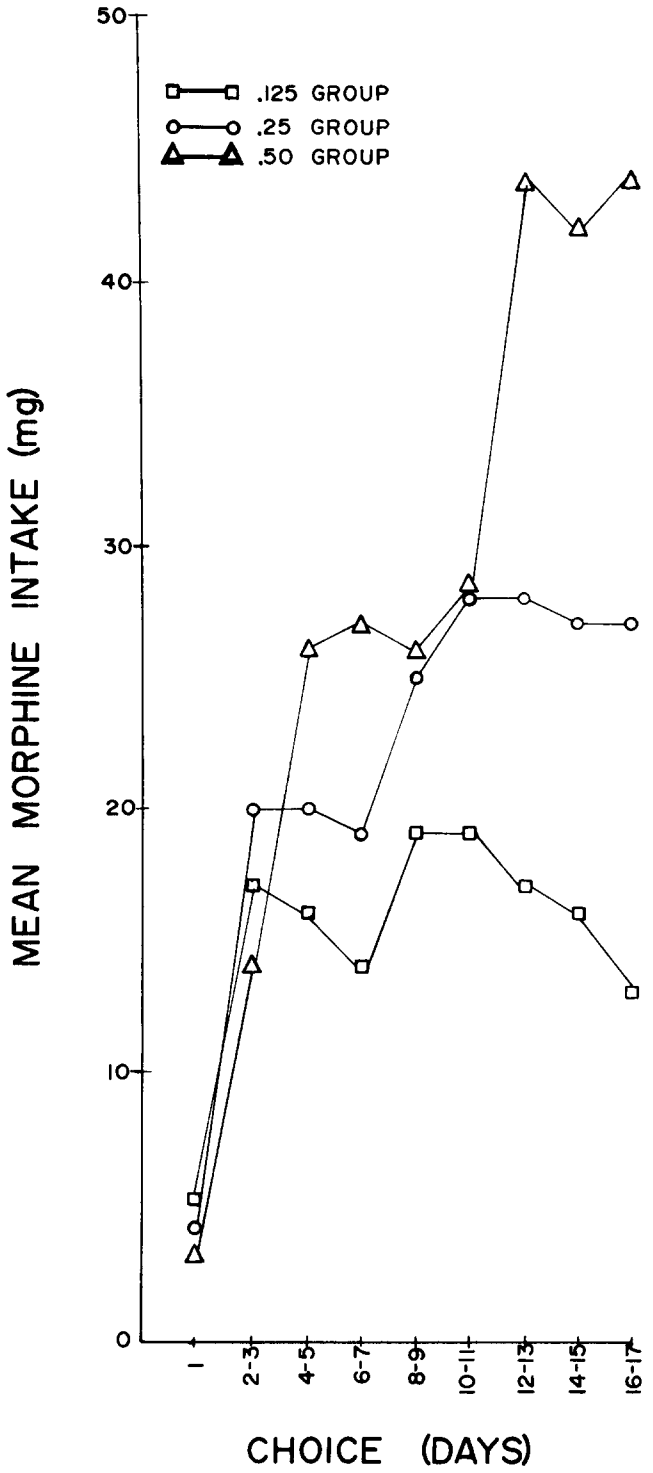

FIG. 2. Mean daily morphine intake for 3 groups of rats during 17 days of choice test where each group had free access to a water and a sucrose-morphine source.

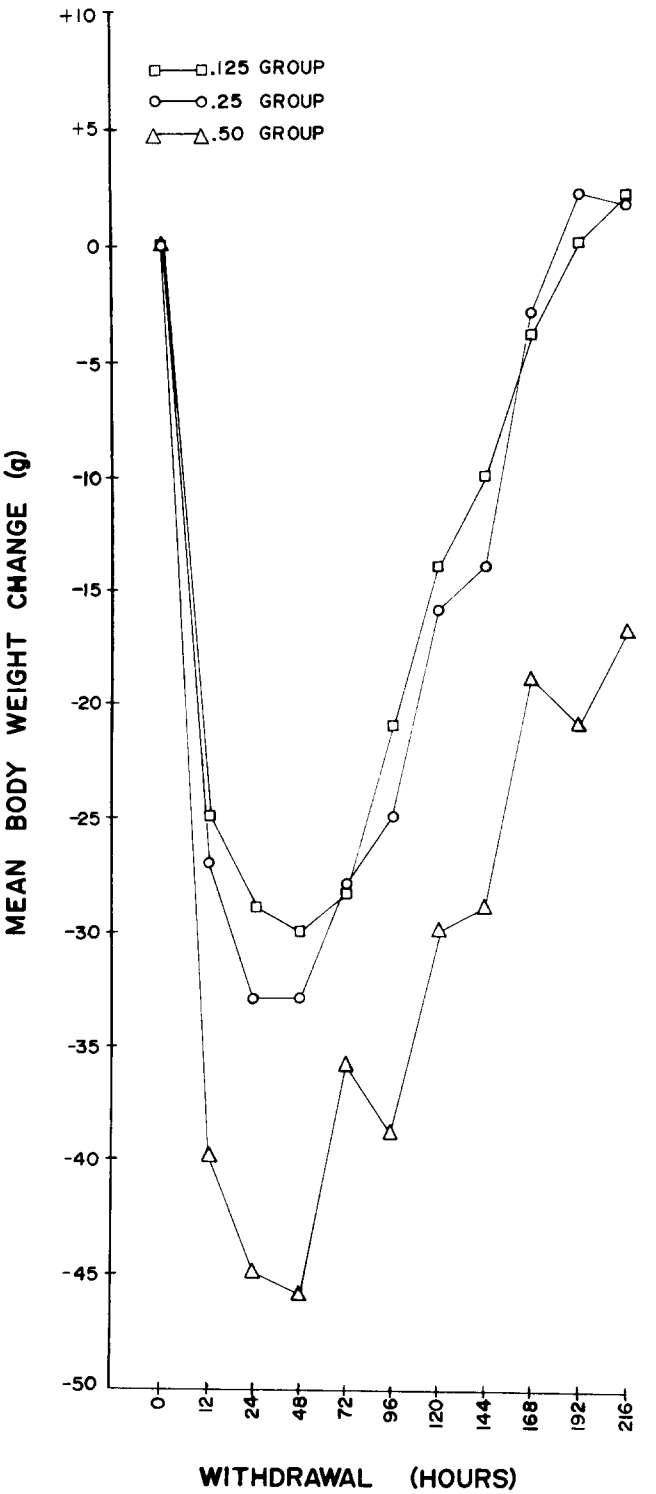

FIG. 3. Mean changes in body weight for 3 groups of rats which had voluntarily consumed sucrose-morphine in preference to water during the immediate preceding 17 days. The sharp drop in body weight was precipitated by a single IP injection of nalorphine and replacement of the animal's sucrose-morphine with the sucrose vehicle.

forced hydration. It can be stated: First, that rats show a clear preference for a 10 percent sucrose solution containing as much as 0.5 mg/ml of morphine HCl over tap water. Second, that there may be an initial aversion with respect to 0.25 and 0.5 mg/ml concentrations that rats overcome rapidly (Fig. 1), and that this aversion is maintained with morphine concentrations in excess of 0.5 mg/ml.

During the next phase of this work we tested for morphine dependence in the above 3 groups. Specifically, we replaced each group's sucrose-morphine with the 10 percent sucrose vehicle and administered an IP injection of a morphine antagonist, nalorphine. Nalorphine administration to a morphine-dependent organism rapidly precipitates morphine abstinence signs which include sharp reduction in eating and drinking, diarrhea, rhinorrhea, pilo-erection, irritability, agitation, and weight loss [6].

Figure 3 shows the effects of the treatment on body weight of the animals. During the first 12 hr after nalorphine administration all animals lost considerable weight. All 3 groups continued to lose weight for an additional 36 hr. The groups which had previously ingested morphine from 0.125 and 0.25 mg/ml in sucrose solutions showed milder abstinence signs in comparison to the 0.5 mg/ml group, as reflected in their recovery of lost body weight (Fig. 3) and food intake (Fig. 4). The 0.5 mg/ml morphine group failed to reach its prenalorphine level of body weight and food intake even after 9 days, while the 2 lower morphine groups recovered their respective baseline levels by the 9th day. This differential recovery from morphine withdrawal signs reflects the fact that the 0.5 mg/ml rats were ingesting considerably more of the drug during the choice phase than the other 2 groups. Other opiate withdrawal signs, mentioned above, were observed in all animals. Diarrhea appeared in all rats within 2 to 5 min after nalophine administration. Piloerection and rhinorrhea were particularly prominent for the first 5 to 6 hr after nalorphine administration.

Previous work in our laboratory had indicated that rats placed on forced morphine sucrose regimen for 25 days prefer a sucrose morphine solution over water – when given a choice. In addition, the same rats also prefer the sucrose vehicle [3]. Recently, we have also obtained data which indicate that rats made morphine-dependent by the above procedure prefer sucrose morphine over water, the sucrose vehicle, or sucrose-adulterated quinine.

All previous studies involving oral self-administration of morphine have employed procedures which involve premedications or forced hydration (by various degrees of either water-depriving the animals or a cyclical method of choice-force alternation [7]. The present work is the first report on the feasibility of making rats dependent on the opiate alkaloid without resorting to forced hydration or premedication.

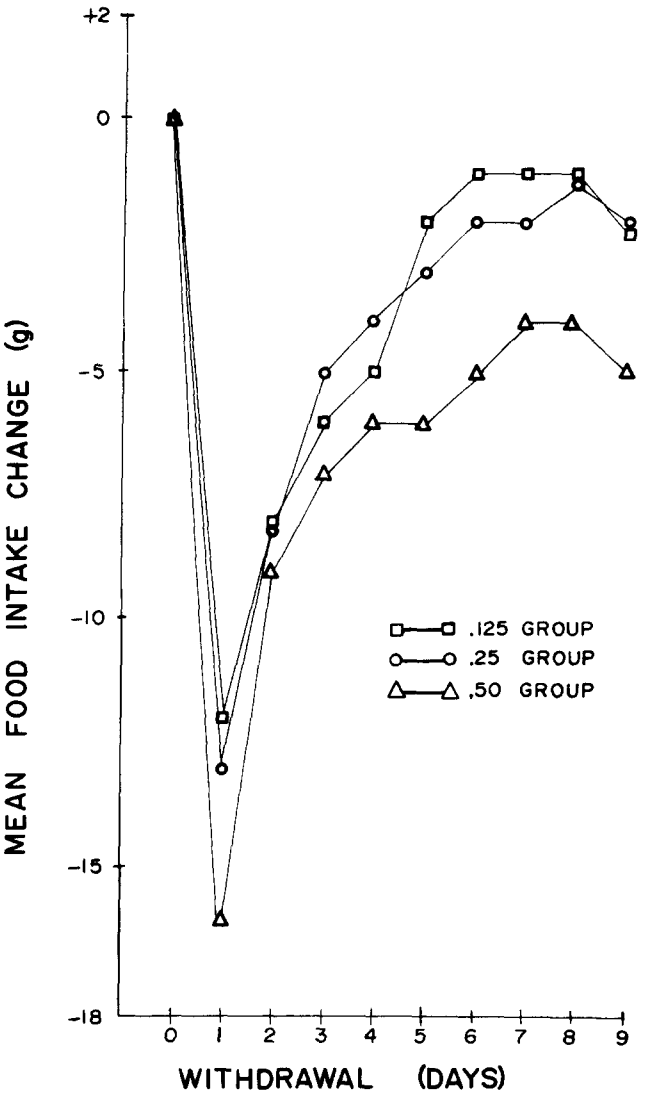

FIG. 4. Mean changes in food intake for 3 groups of rats which had voluntarily consumed sucrose-morphine in preference to water during the immediate preceding 17 days. The sharp drop in eating was precipitated by a single IP injection of nalorphine and replacement of the animal's sucrose-morphine with the sucrose vehicle.

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