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BRIEF COMMUNICATION

# Opioidergic Manipulations Affect Intake of 3% NaCl in Sodium-Deficient Rats

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HUBBELL, C. L. AND N. B. McCUTCHEON. *Opioidergic manipulations affect intake of 3% NaCl in sodium-deficient rats.* PHARMACOL BIOCHEM BEHAV 46(2) 473–476, 1993.—On six weekly occasions, a 3% NaCl solution was presented along with water to rats for 2 h 1 day after being treated with furosemide, a diuretic/natriuretic drug that causes a strong hunger for 3% NaCl. On some of the days, the sodium-hungry rats were injected with morphine in doses ranging from 0.3 to 10.0 mg/kg. Morphine produced biphasic effects on intake of 3% NaCl, with doses of 0.3–3.0 mg/kg increasing intakes dose dependently and 10.0 mg/kg decreasing intakes. The 3.0-mg/kg dose nearly doubled rats' mean intake of 3% NaCl. In contrast, naltrexone, an opioid receptor antagonist, reduced intake of 3% NaCl about 25–40% across doses ranging from 0.1 to 10.0 mg/kg. At some doses of morphine and naltrexone, NaCl ingestion was affected without significant influence of water intake. Therefore, it can be inferred that endogenous opioidergic systems participate in the control of NaCl drinking by sodium-deficient rats. The range of demonstrations of opioid involvement in the control of ingestion can now be extended to the hunger for hypertonic NaCl induced by sodium depletion.

Salt appetite Hypertonic saline intake Ingestion Furosemide Morphine Naloxone Opioids Rats

OPIOIDERGIC manipulations affect the consumption of palatable ingesta (4–6,9–11,18,22,23,26). For example, low doses of morphine, an agonist at opioid receptors, increase rats' intakes of sucrose solutions (11), saccharin solutions (5,6), and sweetened alcoholic beverages (15,16), while naloxone and naltrexone, opioid antagonists, decrease intakes of these same palatable solutions (6,15–17,19,21). Ingestion of certain concentrations of NaCl that are treated as palatable by rats while in sodium balance but thirsty (2,20) is also increased following injections of morphine (3) and by ICV infusions of agonists selective for $\mu$-, $\delta$-, or $\kappa$-opioid receptor subtypes (13,14). Conversely, naloxone reduces intake of NaCl solutions by such rats (8,13).

Highly hypertonic concentrations (e.g., 3% or more) of NaCl are not voluntarily sought and ingested by rats unless they are placed into a state of sodium hunger by sodium-depleting manipulations or hormone injections (12,24). Once depleted of sodium, rats are highly motivated to find and drink solutions that taste like hypertonic NaCl (1,25). Based upon prior work with palatable solutions (mentioned above), it was hypothesized that among sodium-hungry rats low doses of morphine should enhance ingestion of 3% NaCl, while doses of naltrexone should decrease such ingestion.

## METHOD

### *Subjects*

Sixty male Sprague–Dawley rats (Taconic Farms, Germantown, NY) were individually housed in standard hanging cages in a windowless vivarium maintained at 22°C and lighted for 12 h daily beginning at 0700 h. Water was freely available throughout all procedures. Access to food was restricted during specific procedures described below.

### *Apparatus, Drugs, and Solutions*

All testing was performed in rats' home cages. All fluids were presented in glass bottles equipped with ball-point sipping tubes, which prevent substantial spillage. The hypertonic saline solution was 3% NaCl in tapwater.

Morphine sulphate (in 0.9% NaCl) was tested in doses of 0.0, 0.3, 1.0, 3.0, and 10.0 mg/kg. Naltrexone HCl (in 0.9% NaCl) was tested in doses of 0.0, 0.1, 0.3, 1.0, 3.0, and 10.0 mg/kg. Furosemide (in distilled water plus a few drops of NaOH to bring it to pH 7) was administered in two 30-mg/kg doses 2 h apart to induce a strong sodium hunger (25). All injections were given SC in a volume of 1.0 ml/kg.

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*Procedure*

Formal procedures began 4 days after rats arrived at the laboratory and spanned 7 weeks. Across the first 6 weeks, all rats were treated with furosemide, once per week. On each treatment day, rats were given furosemide twice, once at 0900 h and again at 1100 h. In addition, rats were deprived of food beginning at 0900 h on the treatment day until 1100 h the next day to eliminate this source of sodium. On the next day, that is, the test day, at 0900 h all rats were given the opportunity to drink 3% NaCl solution for 2 h. Water was always available. Subsequently, rats were given food and an additional 22 h of access to 3% NaCl to permit complete restoration of body sodium.

All test days were 1 week apart. The first 3 test days constituted the period for establishing baseline. Intakes of water and 3% NaCl solution obtained on the third test day served as the stabilized baseline data.

On the fourth test day, rats were divided into six groups ($n$ = 10) such that the groups' mean g of pure NaCl per kg of body weight taken on the third test day were nearly equal, and then randomly assigned to receive a dose of naltrexone. Naltrexone was given at 0850 h (i.e., 10 min before the test session). The rationale for waiting until the fourth treatment with furosemide before testing naltrexone was to allow rats' consumption of hypertonic saline solutions to stabilize after repeated sodium depletion treatments (16).

On the fifth test day, rats were divided into five new groups ($n$ = 12), with the restrictions that previous history of naltrexone administration was factorially arranged across groups and that the groups' mean g/kg intakes of NaCl during baseline (i.e., the third test) were nearly equal. Doses of morphine were then randomly assigned to groups. Morphine was given at 0845 h (i.e., 15 min before the test session).

The sixth test day served as a postbaseline to determine if there were any carryover effects due to previous naltrexone and morphine histories. The procedures of this test were identical to those of the first three. One week later, the 2-day set of procedures were again performed for the seventh time with the exception that furosemide was not given to rats. This sev-

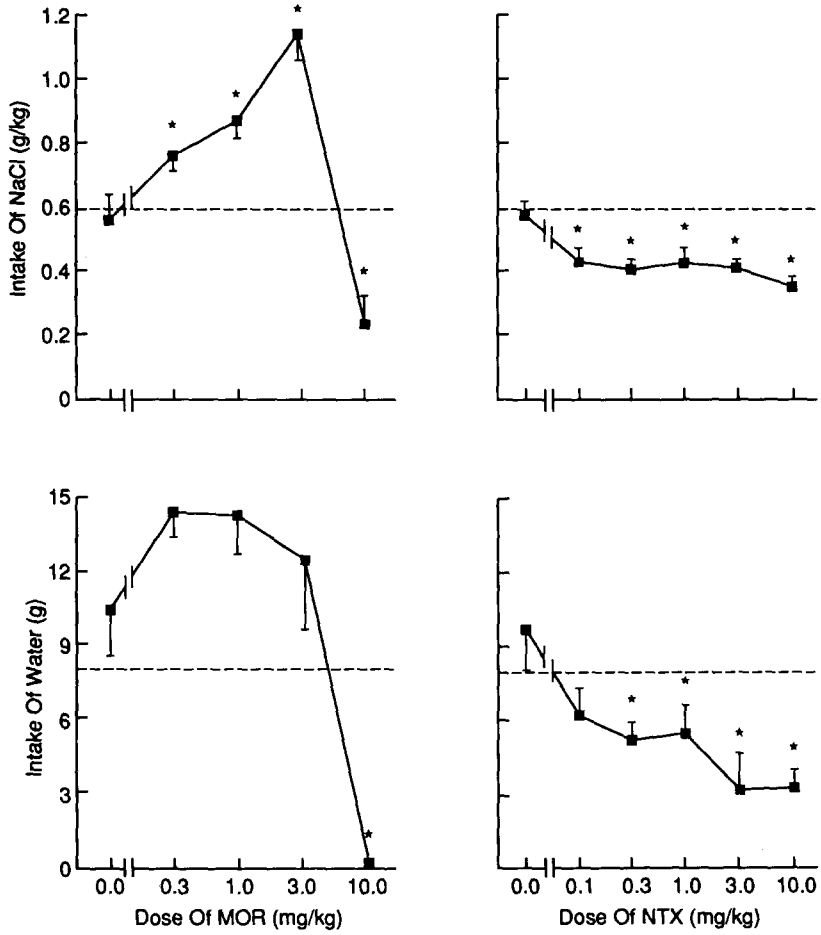


FIG. 1. Dose-response effects of morphine (MOR) and naltrexone (NTX) on mean g/kg intakes of NaCl in 2 h are depicted in the upper left and right panels, respectively. The broken line in each upper panel represents the mean g/kg intakes of NaCl on test day 6 (i.e., furosemide-induced 3% NaCl ingestion 1 week after all opioid testing was completed). Mean grams of water taken in 2 h, under doses of MOR and NTX, are depicted in the lower left and right panels, respectively. The broken lines in the lower panels represent mean intake of water on test day 6. In all panels, error bars represent SEMs and asterisks denote reliable differences from controls (i.e., 0.0-mg/kg doses of each opioid) as discerned by Student's $t$-tests for independent measures.

enth test determined rats' intake of 3% NaCl when not depleted of sodium by furosemide.

*Measures, Data Reduction, and Statistics*

Rats' body weights and intakes of water and 3% NaCl solution during the 2-h test session were tabulated to the nearest 0.1 g. From these basic measures, g of pure NaCl per kg of body weight (g/kg) was derived. This measure, which is highly correlated with g of 3% NaCl solution taken, was used as the best index of NaCl ingestion because it controls for differences in rats' body weights.

As anticipated, initial analyses of the data obtained on the third test day revealed that there were no reliable sources of variance between groups, for any measure, when either the factor of subsequent dose of naltrexone or morphine were used as the grouping variables. Other analyses determined that previous history of naltrexone did not reliably interact with subsequent dosing with morphine.

Intake data on days when opioids were administered conform to one-way analyses of variance (ANOVAs) for the factor associated with groups (i.e., doses of each opioid, five with morphine and six with naltrexone). When reliable sources of variance were revealed, specific between-group comparisons were performed.

The analyses of the data obtained on the sixth test day conform to a 6 × 5 ANOVA with factors associated with previous doses of naltrexone and morphine, respectively. The data of the seventh test day, when rats were not depleted of sodium, were compared to the data of tests 3 and 6 using Student's *t*-tests for dependent measures. These data were also compared to that of the fourth test day, the test with naltrexone. Unless otherwise stated, the intake differences noted in the Results section are reliable at the 0.05 level or better.

## RESULTS

Rats drank more 3% NaCl solution when treated with furosemide than when they were not (0.71 g/kg at test 3 vs. 0.19 g/kg at test 7). The results of testing with morphine, presented in the upper left panel of Fig. 1, show that morphine increased drinking of 3% NaCl among furosemide-treated rats in a dose-dependent manner. Across the 0.3- to 3.0-mg/kg doses of morphine, mean NaCl intakes were increased 36–104% above control (0.0 mg/kg) levels of intake. These doses of morphine produced nonsignificant increases in water intake (see lower left panel of Fig. 1). The 10.0-mg/kg dose of morphine was observably debilitating and led to greatly reduced intakes of both water and 3% NaCl.

Naltrexone affected drinking of 3% NaCl in the opposite manner (upper right panel of Fig. 1). Mean g/kg intakes of NaCl were 26–30% lower among the groups receiving 0.1–3.0 mg/kg naltrexone as compared to controls. The group receiving the 10.0-mg/kg dose took 39% NaCl less than controls (0.0 mg/kg). However, even under the 10.0-mg/kg dose rats took more NaCl (0.35 g/kg) than when not sodium deficient (i.e., at test day 7 all rats took a mean of 0.19 g/kg NaCl). Water intake (lower right panel of Fig. 1) was significantly reduced by all doses of naltrexone except 0.1 mg/kg.

The ANOVA of the data associated with g/kg intake of NaCl on test day 6 yielded $F(20, 30) = 0.55, p > 0.9$, for the interaction term between the factors of previous naltrexone and morphine. Thus, there is no evidence that prior drug history, which was factorially arranged among subjects, produced carryover effects on furosemide-induced 3% NaCl intake at test day 6.

## DISCUSSION

Morphine, in nondebilitating doses, dose relatedly increased intakes of 3% NaCl, to a maximum of around 100% among sodium-hungry rats. It is unlikely that this effect was secondary to morphine causing further loss of body salt or water because in a separate evaluation of this possibility (using rats housed in Nalgene metabolic cages) 3 mg/kg morphine did not lead to measurable increases in furosemide-treated rats' urine excretion during the 2-h period used to obtain intake data in the main study. Water intake was nonsignificantly increased by morphine, a result that may have been secondary to the increased 3% NaCl ingestion.

Naltrexone decreased intake of 3% NaCl solution among sodium-hungry rats by about 30% across all but the highest dose, which resulted in about a 40% reduction. Naltrexone reliably reduced water intake (up to 70%) at all doses except the lowest, a finding concordant with many previous findings (9,21–23). There was, however, some indication that naltrexone's effects were more specific with respect to intake of hypertonic saline because the lowest dose reliably reduced intake of NaCl but not water.

In summary, these data support the conclusion that endogenous opioidergic systems play a role in NaCl ingestion under conditions of sodium depletion where highly hypertonic NaCl solutions gain positive motivational properties. These data add a new motivational condition for ingestion, the hunger for salt, to the body of results showing that opioid manipulations affect ingestion. As suggested before (26), the common factor among all demonstrations of opioidergic involvement in ingestive behavior appears to be an eagerness to ingest a substance regardless of the precipitating conditions for this motivational state.

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