Effects of Levorphanol and Levallorphan on Osmolality of Serum from Water Loaded Rats.* (28622)

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It has been shown by measuring the excretion of a standard water load(1) that tolerance develops to the antidiuretic action of levorphanol administered to rats. In another report(2), using one dose level of levorphanol, measurement of osmolality changes of serum to a water load was used in studying the effect of levorphanol on water balance. The present investigation involves studies on the osmolality of the serum of water loaded rats treated with levorphanol and the effect of levallorphan, an antagonist, on the changes produced.

Methods. Male Sprague-Dawley rats weighing 200 to 300 g were fasted for 18 hours (with free access to water) and treated s.c. (4 ml/kg body weight) with distilled water levorphanol tartrate and/or levallorphan tartrate solution. For brevity, salt forms of the drugs will be referred to respectively as levorphanol and levallorphan. Specific treatment of various groups of rats (number used is in parentheses) was as follows: Group A (35) control injected with distilled water; B (30) 3 mg/kg levorphanol; C (29) 5 mg/kg levorphanol; D (19) 3 mg/kg levorphanol and 1 mg/kg levallorphan; E (19) 3 mg/kg levorphanol and 5 mg/kg levallorphan; F (20) 5 mg/kg levorphanol and 10 mg/kg levallorphan; G (18) 5 mg/kg levallorphan. The time of injection is taken as zero. Immediately preceding drug injection, the rats were gavaged orally with lukewarm tap water, 5% of body weight. At various times the rats were sacrificed by decapitation, blood collected in tubes and allowed to clot. The blood samples were centrifuged and 0.2 ml aliquot of serum transferred to osmometer test tubes. Osmolality was estimated in the Fiske Osmometer. Animals which were sacrificed at time zero were given the s.c. drug injection but were not gavaged with tap water.

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For determining total water content of the stomach and small intestine, female rats were fasted as above and treated as follows: Group 1 (5) control injected with water; 2 (5) 3 mg/kg levorphanol; 3 (5) 5 mg/kg levorphanol; 4 (5) 5 mg/kg levorphanol and 10 mg/kg levallorphan. They were gavaged orally with tap water immediately preceding the drug injections (zero time), sacrificed one hour later and the stomach and small intestine separately ligated and excised. Total water content of each stomach and intestine was estimated by subtracting from the initial wet weight of the organ, the final dry weight. The organ was dried in an oven (about 80°C) and considered to be dry when 2 successive weighings over a 12 hour period changed no more than 0.1 g. These groups were all run in one day.

Results and discussion. In Fig. 1, Panel A, changes in osmolality of the serum to the water load are seen. In the control the water load depressed the osmolality over a 2 hour period, then the values returned to the initial level. With 3 mg/kg levorphanol, the degree of drop of osmolality was the same as in control but the drop persisted for a longer period. This effect was probably due to the antidiuretic effect of levorphanol(1,2) and the absorbed water was retained for a longer period.

The next curve with the 5 mg/kg dose of levorphanol gave a smaller peak drop than either the control or the smaller dose of levorphanol. At the 1 hour point, p was less than .05 (by the "t" test) for the 5 mg/kg levorphanol group compared to control. Persistence of "antidiuretic" effect was maintained in that p was less than .05 comparing the 5 mg/kg group with the control at the 5 hour point.

It was shown previously(2) that a known antidiuretic agent, pitressin, produced the anticipated effects on the osmolality curve. No

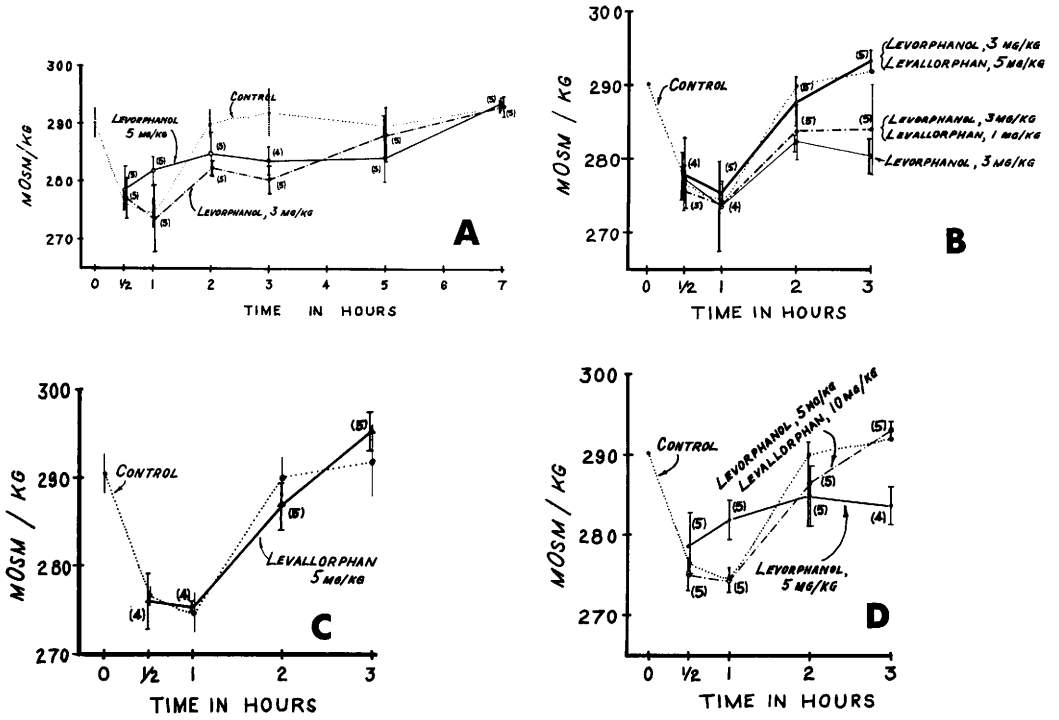

FIG. 1. Effect of levorphanol and levallorphan on changes in serum osmolality in water loaded rats. In control group each point is based on 5 rats. Elsewhere the numbers used are indicated in ( ). Vertical lines indicate standard deviation. A. Effect of 2 different doses of levorphanol on control response. B. Antagonism of effect of 3 mg/kg levorphanol by levallorphan. C. Effect of 5 mg/kg levallorphan alone. D. Antagonism of effect of 5 mg/kg levorphanol by levallorphan.

effect was found on the initial part (first hour) of the osmolality curve. The recovery or ascending limb, however, was so affected that the drop in osmolality was maintained in the pitressin treated group. Since pitressin may be expected to work primarily on the kidney, the results in retrospect may indicate that the initial part of the curve reflects primarily rate of water absorption from the gastrointestinal tract and the ascending limb reflects primarily rate of excretion of water by the kidney. Although this hypothesis is crude (absorption and excretion occur simultaneously) it gave a clue to what might have happened with the 5 mg/kg dose of levorphanol at the 1 hour point. Possibly, the large dose of levorphanol had partially inhibited the absorption of water because the initial part of the osmolality curve was affected.

In Panel B, administration of levallorphan is shown to antagonize the levorphanol effect. Levallorphan was not effective at 1 mg/kg. In Panel C, levallorphan at 5 mg/kg, by itself, had no noticeable effect.

In Panel D, 10 mg/kg levallorphan antagonizes the effect of 5 mg/kg of levorphanol. These results are of particular interest in that the levallorphan antagonized both effects of levorphanol. At 3 hours, the combination is no different from the control. This result indicated that the prolonged osmolality depressant effect of levorphanol was antagonized as in the previous example. The effect of the 5 mg/kg dose of levorphanol at 1 hour was antagonized downward to the control value. Levallorphan antagonized the effect of the levorphanol on both the initial and recovery or ascending parts of the curve.

Partial support for the conjecture that the 5 mg/kg dose of levorphanol inhibited rate of absorption of the oral water load is given in Table I. The total water content of the stomach and intestine from the 3 drug treated and the control group were measured at the

TABLE I. Total Water Content of Stomach and Intestine in Control; 3 mg/kg Levorphanol; 5 mg/kg Levorphanol and 5 mg/kg Levorphanol, 10 mg/kg Levallorphan Groups.

	Water content: mean ± S.E. (g)			
	Control	Levor 3	Levor 5	Levor 5 Leval 10
Stomach	4.5± .6	5.9±1.1	6.5*± .4	5.1± .1
Intestine	10.2±1.1	10.8±1.2	8.8 ±1.0	10.0±1.0

* Indicates $p < .05$ by "t" test. Each value is based on 5 rats.

1 hour point as described in Methods. The only significant difference in water content was found for the stomach of the 5 mg/kg levorphanol group compared to the stomach of the control. This levorphanol treated group retained more water in the stomach than controls, possibly indicating that less water had been absorbed. Levallorphan apparently antagonized the effect of levorphanol. These conclusions must be made with reservation. Had the stomach and intestine been taken together, it appears that no such difference would have been found.

Summary. Levorphanol altered the control serum osmolality response to a 5% oral water load in rats. The change at a small dose of levorphanol was consistent with retention of water systemically owing to the antidiuretic effect of levorphanol. At a high dose, another component appeared in the osmolality curve suggesting that inhibition of water absorption from the gastrointestinal tract was occurring. All effects of levorphanol were antagonized by levallorphan.

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Effects of Hypophysectomy on Steroid-C^{14} Metabolism in the Rat.* (28623)

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The hypophysectomized rat, in contrast to the normal, easily accumulates large concentrations of serum and liver cholesterol when given a dietary supplement of this sterol(1, 2,3). Either a faster rate of absorption or a slower rate of elimination could account for this difference.

The present study investigates the relative turnover of steroid-C^{14} in normal and hypophysectomized rats. Mevalonic acid-2-C^{14} was used as the sterol precursor because this makes it possible to obtain information on steroid-C^{14} synthesis, as well as on rates of tissue β-sterol-C^{14} elimination and fecal steroid-C^{14} excretion. Use of this precursor also circumvents the problem of choosing a suitable cholesterol-4-C^{14} emulsion.

Methods. Female, albino, Sprague-Dawley rats, normal or hypophysectomized, were fed Rockland rat diet *ad lib.* throughout all experiments. Rats were hypophysectomized† at 3 months (215-225 g), after which their weights and CO_2 production were checked periodically for a 4-month period. Eight hypophysectomized rats, with weights plateaued at 185-197 g, were then selected for the experiment. The 8 intact rats used as controls were 7 months old and weighed 250-290 g.

Four of the hypophysectomized and 4 of the normal rats received intraperitoneal injections of 10 μc dl-mevalonic acid-2-C^{14} (1.2×10^6 counts/min./μc as determined by our instruments). One hour later the animals were sacrificed by decapitation, and samples of blood, liver, small intestine, lung, kidney and muscle removed. These tissues and the remaining carcasses were quick frozen, and subsequently analyzed for cholesterol and cholesterol-X-C^{14} concentrations.

The other 4 hypophysectomized and 4 nor-

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