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THE EFFECT OF NALORPHINE ON THE ANTIDIURETIC ACTION OF MORPHINE IN RATS AND MEN

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In several mammalian species the diuretic response to water given by mouth is inhibited by morphine. De Bodo (1944) concluded, from experiments on dogs, that this inhibition cannot be attributed to the effect of morphine on water absorption from the gut; he suggested that it was due to release of posterior pituitary antidiuretic hormone.

Nalorphine (*N*-allylnormorphine) antagonizes some of the pharmacological actions of morphine —for example, its effect on respiration (McCawley, Hart, and Marsh, 1941; Bodman, 1953) and its analgesic action (Unna, 1943; Hart and McCawley, 1944). It seemed of interest, therefore, to see whether nalorphine would antagonize the antidiuretic effect of morphine, and whether this could be achieved without affecting its analgesic action.

METHODS

Effects on renal water excretion in rats were tested by a method similar to that described by Ginsburg (1951). Adult male albino rats weighing approximately 200 g. were used. They were deprived of food for 16 hr. before the test, but were allowed free access to water. Each animal was then weighed, given water equivalent to 5% of its body wt. by stomach tube and placed in a metabolism cage. One hr. later the animals were injected subcutaneously with the drug or drugs to be tested, and were again given water equivalent to 5% of their body wt. The volumes of urine excreted in the first hr. were noted. The "water load" of the animal at the beginning of the second hr. could thus be calculated. Urine volumes were noted at 30 min. intervals for the following 3 hr. Immediately before each reading the animals were made to empty their bladders, by prodding or stroking. Cross-over experiments were done with at least a week's interval between tests. Morphine sulphate and nalorphine hydrobromide dissolved in 0.9% NaCl solution were used. Intestinal water absorption was measured by the method of Heller and Smirk (1932). Analgesic effects in rats were estimated by the time required from exposure to a standard thermal stimulus until the rat moves its tail (Davies, Raventos, and Walpole, 1946). To avoid injury the animal's tail was never exposed to the stimulus for more than 20 sec.

The water content of the rat brains was determined as follows. Two groups of adult male rats of 180–210 g. were treated as in the diuresis tests. One group was injected with 0.9% NaCl solution and the other with morphine sulphate (10 mg./kg.). At 0, 90, 180, and 270 min. after the injection, rats from both groups were quickly anaesthetized with ether and killed by bleeding. The brain was carefully dissected, placed on a weighed watchglass, and weighed. The cerebellum, cerebral hemispheres, medulla, and pons were immediately dissected from the basal nuclei and each of these pieces of brain weighed separately. The dissected brains were then dried.

Water diuresis in man was investigated as follows: The subjects were deprived of food and fluids for 12 hr. before the test. They then emptied their bladders and had the drug or drugs injected intramuscularly. Immediately afterwards they drank a standard volume of water (860 ml./m.^{2} body surface). They lay on couches during the test, but were allowed to stand up to pass urine. In one instance a subject was not allowed to sit or stand during the test. Urine was collected at hourly intervals. Whenever possible cross-over experiments were performed with at least a week's interval between tests. All volunteers were unaware of what drug or drugs they received, and the drugs were given in a random manner.

RESULTS

Antidiuresis in Rats.—Fig. 1 shows the marked antidiuretic effect of a subcutaneous injection of 10 mg./kg. morphine sulphate in rats. When this dose of morphine sulphate (=MO) was injected simultaneously with nalorphine hydrobromide (=NA), in the ratio of 1:2.5, there was a marked diminution in the antidiuretic response. When, however, the procedure was repeated but the ratio MO:NA was 12:1 there was no significant difference in the antidiuretic response. When the ratio MO:NA was 6:1 there was a slight diminution in the antidiuretic effect: rats, given morphine

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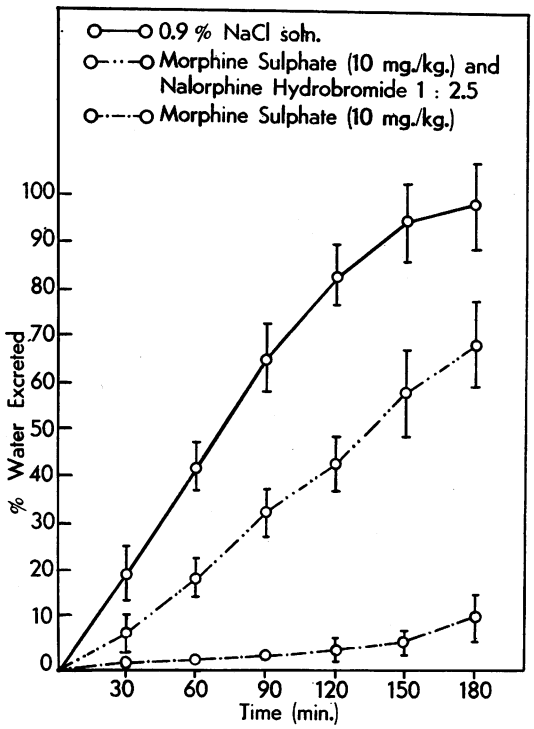

FIG. 1.—The effect on the antidiuretic action of morphine sulphate in rats of the simultaneous injection of nalorphine hydrobromide and morphine sulphate in the dose ratio MO: NA = 1: 2.5.

(10 mg./kg.) and nalorphine simultaneously in the ratio of 12:1, excreted 24.7 ± 1.4% (mean ± S.E.) of the water load in 3 hr., whereas when the ratio was 6:1 the excretion was 41.2 ± 4.7% in a similar time. Rats given morphine sulphate only, excreted 23.8 ± 0.8% of their water load in 3 hr.; the control animals injected with 0.9% NaCl solution excreted 92.1 ± 9.1% of their water load during the same period.

Similar experiments were done to see whether nalorphine by itself had any antidiuretic effects in rats. There was no significant difference between rats given nalorphine hydrobromide (0.8 mg./kg. or 25 mg./kg.) subcutaneously and controls given 0.9% NaCl solution. The rats given nalorphine excreted 96.0 ± 11.2% (mean ± S.E.) of the water load in 3 hr. as against 99.9 ± 6.6% (t = 0.56 P > 0.5) in the controls. Rats given 25 mg./kg. nalorphine HBr excreted 90.2 ± 8.2%, and their controls excreted 99.0 ± 7.6% (t = 0.88 P > 0.4).

Table I shows the effect of morphine, nalorphine, and of the two drugs combined, on gastro-intestinal water absorption 180 min. after injection. There was a significant difference between the mean weight of the gastro-intestinal tracts of the group of rats injected with morphine and that of the control group. This was mainly due to differences in stomach weight. Pyloric spasm was seen in many of the morphine-treated animals when the abdomen was opened 180 min. after the administration of water. Similar results were obtained 90 min. after the administration of water and drug. However, even if both the increase in gastric emptying time and the decrease in the rate of intestinal water absorption are taken into account, and urine volume is expressed as % of absorbed water excreted, rats which received morphine sulphate and nalorphine hydrobromide in a dose ratio of 1:2.5 excreted more of the administered water than the animals which received morphine only (Fig. 2).

Analgesia in Rats.—Table II shows the reaction time of rats to a standard thermal stimulus at various intervals after the injection of nalorphine, morphine, or a combination of these drugs. The rats given 0.9% NaCl solution maintained much the same reaction time (8–10 sec.) during the course of the test; animals given morphine showed marked analgesia (reaction time uniformly >20 sec.); nalorphine (25 mg./kg.) alone had a slight analgesic effect most marked after about 40 min. Although analgesic effects could also be demon-

TABLE I

THE EFFECT OF NALORPHINE, OF MORPHINE, AND OF MORPHINE AND NALORPHINE SIMULTANEOUSLY, ON THE WT. OF THE RAT'S INTESTINAL TRACT AFTER ADMINISTRATION OF WATER

(The numerals are the means ± S.E. from groups of 6 animals. Numerals in parentheses are % changes)

Treatment	After 90 min.			After 180 min.		
	Wt. of Stomach (g.)	Wt. of Intestine (g.)	Wt. of Whole Gastro-intestinal Tract (g.)	Wt. of Stomach (g.)	Wt. of Intestine (g.)	Wt. of Whole Gastro-intestinal Tract (g.)
0·9% NaCl soln.	5·9 ± 0·1	10·2 ± 0·1	16·1 ± 0·4	5·9 ± 0·1	10·2 ± 0·1	16·1 ± 0·1
Morphine sulphate, 10 mg./kg.	7·1 ± 0·2 (+20)	10·5 ± 0·6 (+3)	17·6 ± 0·7 (+9)	6·9 ± 0·1 (+17)	10·9 ± 0·2 (+7)	17·8 ± 0·2 (+11)
Nalorphine hydrobromide, 25 mg./kg.	6·5 ± 0·1 (+10)	10·5 ± 0·1 (+3)	17·0 ± 0·1 (+6)	6·0 ± 0·1 (+2)	10·1 ± 0·3 (−1)	16·2 ± 0·4 (+1)
Morphine sulphate: nalorphine hydrobromide, 1:2·5	6·5 ± 0·2 (+10)	10·4 ± 0·1 (+2)	17·0 ± 0·5 (+6)	6·4 ± 0·1 (+9)	10·0 ± 0·1 (−2)	16·4 ± 0·1 (+2)

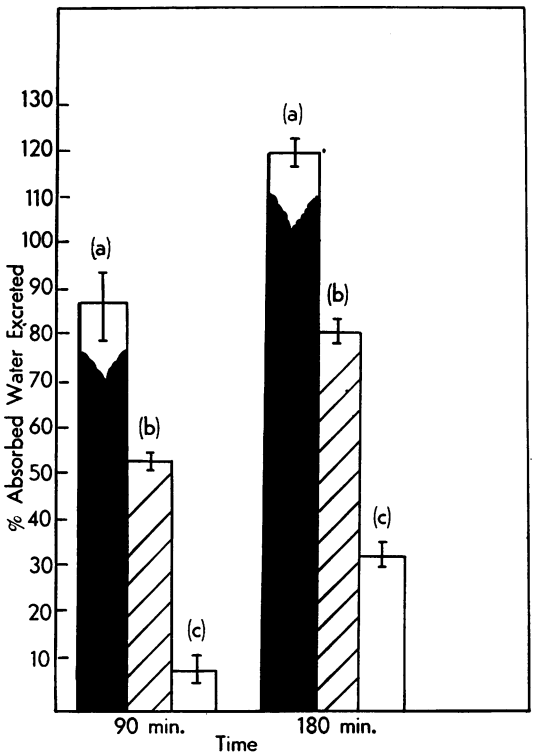

FIG. 2.—Comparison of the effect of subcutaneous nalorphine hydrobromide and morphine sulphate given simultaneously, with that of morphine sulphate alone, on the percentage of absorbed water excreted 90 min. and 180 min. after administration of the water. (*a*) 0.9% NaCl soln.; (*b*) morphine sulphate (10 mg./kg.): nalorphine hydrobromide, 1:2.5; (*c*) morphine sulphate (10 mg./kg.).

TABLE II

REACTION TIMES (SEC.) OF RATS TO A STANDARD THERMAL STIMULUS BEFORE AND AFTER VARIOUS TREATMENTS

(All rats were given 10% of their body weight of water in two divided doses in addition to the NaCl soln. or the drugs. The numerals are the means ± S.E. Numerals in parentheses are no. of animals per group.)

Treatment	Before Injection	Min. after Injection		
		40	80	155
NaCl 0·9% soln. ..	9·6±0·6 (11)	9·8±0·7 (10)	7·6±0·6 (10)	9·1±0·9 (7)
Morphine sulphate 10 mg./kg.	7·6±0·7 (11)	All >20 (10)	All >20 (10)	All >20 (7)
Nalorphine hydrobromide, 25 mg./kg.	10·4±1 (10)	10, >20, 9, 12, 13, 10, 19, 17, >20, 13*	12·1±1·3 (10)	7, 15, 10, 9, 12, >20, 11*
Morphine sulphate: nalorphine hydrobromide 1:2·5	10·9±0·9 (10)	18, >20, 11, >20, 14, 11, 5, 20, 17, 16*	>20, 14, >20, 11, 7, 13, 8, 10, 17*	>20, 9, >20, >20, 12, 16, 12*

* Reaction times of individual rats.

strated in animals given morphine sulphate and nalorphine hydrobromide, the morphine analgesia was diminished by the nalorphine. The dose of nalorphine which diminishes the antidiuretic effect of morphine in rats also, therefore, weakens its analgesic action.

Antidiuresis in Man.—The effects of morphine, nalorphine and combinations of these drugs on water diuresis were also tested in healthy men aged from 19–35. Nalorphine alone (9.2 mg./m.²) was given to 5 subjects. The results were compared with those of control experiments in which, a week before or after the experiment, the subject received an injection of 0.9% NaCl solution. Two types of response to nalorphine were observed: three men showed no inhibition of their renal response to water, but two showed a pronounced inhibition of water diuresis (Fig. 3). The inhibition could not be correlated with clinical signs and symptoms, for nalorphine, in the dose used, produced pallor, giddiness, mild euphoria, and disorientation in all the subjects.

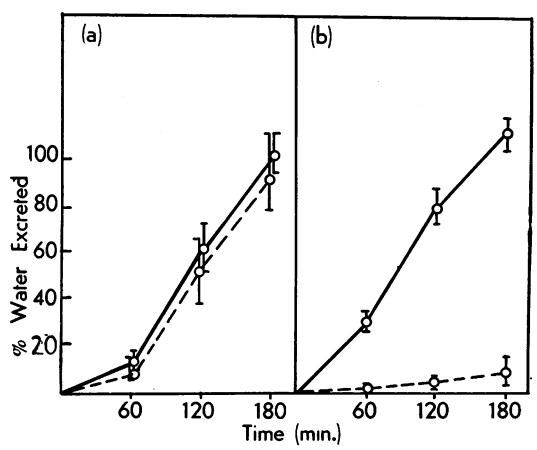

FIG. 3.—Effect of intramuscular nalorphine hydrobromide (9.2 mg./m.²) on the water diuresis of healthy human subjects. (*a*) Mean results in 3 subjects; (*b*) mean results in 2 subjects. Continuous line, 0.9% NaCl solution. Broken line, nalorphine hydrobromide, 9.2 mg./m.²

Morphine sulphate (9.2 mg./m.²) produced a variable degree of inhibition of water diuresis in all the subjects investigated. After morphine, five subjects had excreted 33.4 ± 13.7% (mean ± S.E.) of the water load after 3 hr., whereas after normal saline the same subjects had excreted over 100% (105.4 ± 0.4) of the water load (Fig. 4). In one subject, given morphine sulphate (9.2 mg./m.²) 45 min. after administration of water, and not allowed to get up during the test, inhibition of

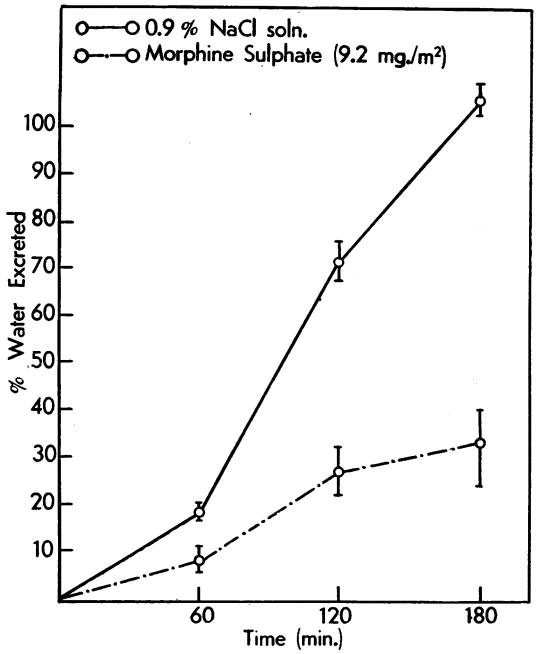

FIG. 4.—Effect of 9.2 mg./m.² morphine sulphate on the water diuresis of 5 healthy men.

water diuresis was also observed. He excreted 7% of his water load in 3 hr. after morphine, compared with 92% after 0.9% NaCl solution.

Side Effects in Man.—The toxic side effects of morphine seemed to be delayed by the administration of the water load. All subjects were drowsy and one subject vomited. The drowsiness became more marked between 3 and 9 hr. after injection, and several subjects vomited frequently. One man developed marked respiratory depression during this period. The subjects who showed the most marked delay in water excretion also exhibited the most marked toxic symptoms between the 3rd and 9th hours. Further tests on human volunteers thus seemed inadvisable. In contrast, however, to the results in rats, nalorphine and morphine in the dose ratio 1:1 did not increase the renal response of the subjects to water (Table III).

In similar experiments using morphine combined with nalorphine, in the ratio 1:2.5 (Table III), antidiuretic effects were still observed. These subjects showed toxic effects such as giddiness, euphoria and nausea.

It was thought that the delayed toxic effects of morphine were due to cerebral oedema. To test this hypothesis the effect of morphine sulphate on the water content of the brains of rats was investigated.

TABLE III

HEALTHY MEN. EFFECT ON WATER DIURESIS OF COMBINED TREATMENT WITH MORPHINE SULPHATE (MO) (9·2 MG./M.²) AND NALORPHINE HYDROBROMIDE (NA), COMPARED WITH MORPHINE SULPHATE ALONE

Subject and Age (yr.)	Body Weight (kg.)	Surface Area (m.²)	Drug and Dose (mg.)	% Water Load Excreted in 3 hr.
	A. Dose ratio MO: NA = 1: 1			
R.B. (27)	63	1·75	MO 16·1 NA 16·1	34
R.B. (27)	63	1·75	MO 16·1	29
C.W. (34)	95	2·15	MO 19·8 NA 19·8	25
C.W. (34)	95	2·15	MO 19·8	63
J.M. (35)	70·2	1·81	MO 16·7 NA 16·7	15
J.M. (35)	70·2	1·81	MO 16·7	46
	B. Dose ratio MO: NA = 1: 2·5			
E.J. (19)	66·5	1·75	MO 16·1 NA 40·3	2·0
E.S. (19)	71·4	1·8	MO 16·6 NA 41·5	24·0
R.K. (23)	65·4	1·75	MO 16·1 NA 40·3	81·0
J.A. (23)	65·2	1·78	MO 16·4	78·0
H.S. (27)	65·4	1·85	0·9% NaCl	107·0

Table IV shows the water content of the brains 0, 90, 180 and 270 min. after the injection of 10 mg./kg. morphine sulphate. No significant difference from the controls could be detected. The same method gave similarly negative results when the water content of the cerebral hemispheres, basal nuclei, medulla and pons, and cerebellum were estimated separately.

TABLE IV

EFFECT OF MORPHINE SULPHATE ON THE BRAIN WATER CONTENT OF HYDRATED RATS (EXPRESSED AS % WT. OF BRAIN)

(Mean ± standard error from groups of 5 animals)

Time after Injection (min.)	NaCl Soln. (0·9%)	Morphine Sulphate (10 mg./kg.)
0	44·2 ± 1·6	43·8 ± 1·4
90	43·8 ± 1·4	42·8 ± 1·7
180	44·6 ± 0·2	44·3 ± 1·2
270	43·4 ± 1·9	41·4 ± 1·0

DISCUSSION

Nalorphine may decrease the antidiuretic effect of morphine in several ways. An action of morphine on the posterior pituitary is suggested by the fact that, in dogs, direct injection of morphine into the supra optic nucleus produces an antidiuresis (Duke, Pickford, and Watt, 1951), and that morphine does not inhibit urine flow in "neurohypophysectomized" animals (De Bodo, 1944). Large doses, however (2 mg./kg. i.v.), also influence glomerular filtration rate (Handley and Keller, 1950). Similarly Giarman, Mattie and Stephenson (1953) found more antidiuretic

activity in the urine of intact rats given morphine than in hypophysectomized animals. Lipschitz and Stokey (1947), on the other hand, reported that renal denervation lessened the antidiuretic effect of morphine. It seems possible, therefore, that nalorphine prevents, or decreases, the release of the pituitary antidiuretic principle by morphine in the rat. However, since no measurements of renal plasma flow and glomerular filtration rate were done, another and perhaps additional mechanism—namely, antagonism to the renal vascular effects of morphine—cannot be excluded.

De Bodo (1944) found no impairment of water absorption from the gastro-intestinal tract in dogs given morphine. This seems, at first sight, to be at variance with our results. Apart from the difference in species, however, de Bodo injected the morphine 40 min. after the administration of water, whereas in our experiments on rats the morphine and water were given simultaneously. Hart and McCawley (1944) showed that nalorphine (0.5 mg./kg.) decreased the effect of morphine (1.0 mg./kg.) on the tone of the gut in unanaesthetized dogs with Thiry Vella loops. Similar results were obtained in man (Beal and Shapiro, 1953) and in unanaesthetized dogs with modified Mann loops (Gruber and Gruber, 1953). The effect of nalorphine in decreasing the action of morphine on the water absorption in rats may thus be due to a gastro-intestinal effect.

Hart and McCawley (1944) believe that nalorphine is at least as potent as morphine in raising the pain threshold of rats. Unna (1943), in mice, and Winter, Orahovats, Flataker, Lehman, and Lehman (1954), in rats, concluded that the analgesic effect of nalorphine was much lower; our results agree with the latter conclusion.

The fact that nalorphine in a dose which produced a moderate decrease in the antidiuretic effect of morphine in rats also weakened its analgesic effect, and that nalorphine in doses of 9.2 mg./m.^{2} and 23 mg./m.^{2} failed to have any appreciable effect on the antidiuretic action of morphine in the few human subjects used, indicate that it would be of little clinical use in preventing antidiuresis from morphine.

It is well known that the analgesic response to a standard dose of morphine varies considerably in human beings. Nalorphine has many of the actions of morphine though to a lesser degree. One would therefore expect that the individual response to nalorphine would vary similarly. Moreover, those subjects who did not show the antidiuretic effect of nalorphine after the administration of water would probably have done so if the dose had been increased. However, larger doses could not be given since, even with the dose used, all subjects showed mild toxic effects.

Whether morphine has an antidiuretic effect in human beings is still a controversial issue (Table V), but antidiuresis was seen in all of our experiments. It may have been due to diminution

TABLE V

EFFECT OF MORPHINE SULPHATE ON THE RENAL RESPONSE OF HUMAN BEINGS TO WATER

Authors	Subjects	Posture	Dose and Route of Admin.	Renal Response to Water Load
Fee (1928) ..	Male adult	Not stated	16 mg. s.c.	Diminished
Bahn, Iserbeck, and Linderman (1930)	Patients with sciatica and nephrolithiasis	,, ,,	20 ,, ,,	,,
Ferrer and Sokaloff (1947)	Patients (convalescent)	,, ,,	10–16 mg. i.m.	,,
Walker (1949)	Students Male	,, ,,	20 mg. s.c.	Unchanged
Lewis (1953)	Patient	Recumbent	5·4 mg. i.v.	,,
		Standing		Diminished
Schnieden and Blackmore (this paper)	Healthy male subjects	Recumbent	16·1 mg. i.m.	,,
		Standing	16·1–18·4 mg. i.m.	,,

of renal blood flow or stimulation of the posterior pituitary. A decrease in urine flow still occurred in a subject kept horizontal, so standing cannot be an important factor. Decreased water absorption also can only be contributory, since morphine inhibited urine flow, even when 45 min. were allowed for water to be absorbed. Spurious antidiuresis due to action of morphine on the sphincter muscle of the bladder could also be excluded.

The ability of nalorphine to diminish the "antidiuretic" effect of morphine in man may be a question of the dose ratio. In rats, nalorphine HBr, administered with morphine sulphate in a dose ratio MO:NA=12:1, caused no difference in the renal response to water compared with animals given morphine only. When the dose ratio MO:NA was raised 1:2.5, the antidiuretic effect of morphine was markedly diminished. Since, however, the highest dose of nalorphine hydrobromide used in man (42 mg./m.^{2}) already produced slight toxic effects, it seemed undesirable to raise the dose further.

The delayed toxic effects of morphine sulphate (9.2 mg./m.^{2}) may conceivably have been due to the extra water load, but it should be pointed out that this was never greater than 2% of the body weight. Ellerbrook, Dunham, and Barbour (1930)

have shown that massive doses of vasopressin injected into hydrated rabbits caused oedema of the brain. It was therefore thought that the following sequence of events may have produced the toxic effects in human beings: stimulation by morphine of the neurohypophysis, increased release of antidiuretic hormone, and oedema of the brain leading to vomiting, drowsiness, and respiratory depression. There was, however, no difference between the water content of various parts of the brain in hydrated rats given morphine and that of control rats given 0.9% NaCl.

Summary

1. The antidiuretic effect of morphine in rats is diminished by the simultaneous injection of a dose of nalorphine which alone produces no antidiuretic effect.

2. The effect of morphine is due partially to an action on gastric emptying time; simultaneous administration of nalorphine partially counteracts this effect. After water has been absorbed, morphine inhibits its excretion. Simultaneous administration of nalorphine diminishes this effect.

3. Nalorphine reduces the analgesic effect of morphine. Nalorphine itself produces slight analgesia.

4. The water content of the brain of rats to which water has been administered is not significantly higher after morphine than after saline.

5. Morphine has a marked antidiuretic effect on healthy men given water. Nalorphine produces an antidiuresis in some subjects.

6. Nalorphine injected simultaneously with morphine does not prevent the occurrence of marked antidiuretic effects in men.

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