Pharmacology Biochemistry & Behavior, Vol. 15, pp. 109–114, 1981. Printed in the U.S.A.

Suppression of Drinking by Naloxone in Rats Homo- and Heterozygous for Diabetes Insipidus[1]

DAVID R. BROWN[2] AND STEPHEN G. HOLTZMAN[3]

Department of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322

Received 25 November 1980

BROWN, D. R. AND S. G. HOLTZMAN. *Suppression of drinking by naloxone in rats homo- and heterozygous for diabetes insipidus*. PHARMAC. BIOCHEM. BEHAV. **15**(1) 109–114, 1981.—The effects of the opiate antagonist, naloxone, alone and in combination with morphine, were examined on drinking induced by water deprivation in homo- and heterozygous Brattleboro rats manifesting an inherited diabetes insipidus. Both naloxone and a structurally-related congener, naltrexone (0.01–10 mg/kg), attenuated water consumption in a dose-related fashion of 1 hr water-deprived homozygotes, which exhibit a complete absence of vasopressin. Drinking was also reduced by the two drugs in 24 hr water-deprived heterozygotes, which have detectable levels of vasopressin. Morphine pretreatment enhanced the antidipsogenic effects of naloxone in a dose- and time-dependent manner. The administration of 10 mg/kg of morphine 3 hr before testing, which itself did not affect drinking, maximally potentiated the suppressant effects of naloxone on drinking in homozygotes. This potentiating effect of morphine persisted for at least 48 hr. These results indicate that vasopressin is not essential for the antidipsogenic effects of the narcotic antagonists. The polydipsic Brattleboro rat may provide a convenient animal model for studies of the effects of opiate agonists and antagonists on drinking behavior.

Naloxone Naltrexone Water deprivation Diabetes insipidus Water intake Vasopressin Brattleboro rat

NALOXONE, a pure opiate antagonist devoid of significant intrinsic activity at low doses [4,32], has become a useful pharmacological tool in studies designed to determine the possible physiological functions of the endogenous opioid peptides (e.g. endorphins and enkephalins) [15]. The drug produces numerous physiological and behavioral effects, presumably through its blockade of opiate receptors and a consequent disruption of activity in endogenous opioid pathways [15, 19, 36].

A number of recent investigations have disclosed that naloxone exerts profound effects upon water intake, particularly that which occurs in response to conditions of actual or simulated body fluid deficits. In the rat, naloxone suppresses water consumption induced by periods of water deprivation [5, 6, 13, 16, 17, 25, 34], and by systemic injections of hypertonic sodium chloride solutions, which produce an intracellular dehydration [6,10], as well as drinking stimulated by isoproterenol and angiotensin II, which mimic states of extracellular dehydration [7]. In contrast, schedule-induced drinking, generated by intermittent food delivery and occurring in the absence of fluid deficits, is not attenuated by low to moderate doses of naloxone [7,23].

In the study of drug effects on drinking behavior, the Brattleboro rat may provide a convenient experimental model. Rats of this strain manifest a severe hypothalamic diabetes insipidus due to an inherited deficiency in the synthesis of vasopressin [22,40]. Vasopressin, a peptide hormone synthesized in hypothalamic magnocellular neuroendocrine cells and released from the posterior pituitary, is intimately involved in the control of water metabolism, principally through its antidiuretic action [30]. Animals homozygous for the disease exhibit a complete lack of vasopressin in the plasma [28] and brain [14] resulting in a marked polyuria with impairments in urine-concentrating ability and an intense polydipsia [22]. Furthermore, homozygous Brattleboro rats appear to manifest genetic defects in the opiate system. For example, these animals display an impaired development of tolerance to the analgesic effects of morphine [11], decreased levels of leucine-enkephalin-like immunoreactivity in the pituitary [31] and alterations in opiate receptor number and affinity in tel- and diencephalic regions of the brain [29].

In light of these many characteristics, one objective of this investigation was to evaluate the effects of naloxone and

[1]This investigation was supported in part by USPHS grants 5T01 GM00179, DA00541 and by Research Scientist Development Award KO2 DA00008 to S. G. H.

[2]Present address: The University of Chicago, Department of Pharmacological and Physiological Sciences, 947 East 58th Street, Chicago, IL 60637.

[3]To whom reprint requests should be addressed.

a structurally-related congener, naltrexone, on drinking induced by water deprivation in Brattleboro rats both homozygous and heterozygous for diabetes insipidus. Heterozygotes manifest reduced plasma and brain vasopressin levels, but their water consumption lies within the range of normal rats [14, 28, 41]. In addition, several reports have indicated that pretreatment with single doses of opiate agonists in mice markedly enhances the potency and efficacy of the narcotic antagonists in reducing the analgesic effects of morphine [35, 37, 38, 43]. It was of interest to apply these findings to the actions of naloxone in the present drinking paradigm. Therefore, another objective was to determine if pretreatment with single doses of morphine, having no effects on drinking alone, would modify the ability of naloxone to suppress water intake of the homozygous Brattleboro rat.

METHOD

Animals

The subjects were male Brattleboro rats: 21 homozygous and 12 heterozygous for diabetes insipidus (Blue Spruce Farms, Altamont, NY). All animals weighed between 160–230 g at the start of the experiments. The subjects were housed in individual cages in a colony room with an average temperature of 72°F and under constant illumination between 0700–1900 hr. Water and food (Rodent Laboratory Chow No. 5008, Ralston Purina Co., St. Louis, MO) were freely available in the home cages except during periods of controlled water deprivation.

Narcotic Antagonist Studies

Twelve rats heterozygous and 6–9 homozygous for diabetes insipidus were used in studies to characterize the suppressant effects of naloxone and naltrexone on drinking. In these and all subsequent experiments, homo- and heterozygotes were deprived of water for 1 and 24 hr, respectively, prior to tests of water intake. Food was present during all water deprivation intervals. Every subject underwent 2–3 trial sessions for familiarization with the deprivation and experimental procedures before the initiation of drug testing. Thirty minutes prior to water intake determinations, rats were weighed and injected SC with drug (0.01–10 mg/kg) or isotonic saline. Tests with naloxone preceded those with naltrexone. In studies of the time course of action of the narcotic antagonists, a single 3.0 mg/kg dose of naloxone or naltrexone was administered SC to 1 hr water-deprived homozygotes. Drug injections were given on separate days, at randomly-determined 30 min intervals up to 6 hr prior to water intake measurements. In these studies only, control injections with isotonic saline were administered at the start of the water intake test period. Subjects in all studies were allowed free access to water in the home cages for 30 min from a 25 ml graduated cylinder fitted with a metal drinking spout. Water intake tests were conducted in both the presence and absence of food; food was absent in time course studies. Water intake at the end of the test period was measured to the nearest 0.2 ml. Following drinking tests, animals were given free access to food and water until the next period of deprivation. All tests were conducted between 0900–1500 hr, twice weekly in heterozygotes and three times weekly in the homozygotes.

Morphine Pretreatment Studies

The effects of morphine on the ability of naloxone to suppress drinking in homozygous Brattleboro rats were examined in two sets of experiments. In one study, the effects of morphine were evaluated with respect to dose in 11 homozygotes. Rats were pretreated with morphine (0.1–17.5 mg/kg) or isotonic saline, SC, 3 hr prior to water intake tests. Naloxone (1.0 mg/kg) or saline was injected SC 30 min before testing. Water consumption was measured in the home cages for 30 min, in the manner described above. Food was absent during the tests of water intake. In a second study, the effects of morphine were examined as a function of the length of the pretreatment interval preceding naloxone administration. This study was conducted in 9–11 homozygote rats. Morphine (10 mg/kg) or saline was injected SC, 3, 12, 24, or 48 hr prior to water intake determinations. Naloxone (0.01–10 mg/kg) or saline was administered SC, 30 min before testing. Water consumption was measured in the home cages for 30 min, in the manner described previously. Food was present during the tests of water consumption. Testing in both studies was conducted three times weekly between 0900–1300 hr.

Drugs

Naloxone hydrochloride, naltrexone hydrochloride (both supplied by Dr. Robert E. Willette, National Institute on Drug Abuse), and morphine sulfate (S.B. Penick Co., Newark, NJ) were dissolved in 0.9% saline and injected subcutaneously in a volume of 1.0 ml/kg of body weight. In each series of experiments, the various doses of naloxone and naltrexone (0.01–10 mg/kg) and morphine (0.1–17.5 mg/kg) were administered in random sequence. All drug doses are expressed in terms of the free base.

Data Analysis

Water intake data were converted to ml of water consumed per kg of body weight. In order to facilitate comparisons among the different experimental groups, the data were further transformed to a percentage of the saline control value for each animal in each series of experiments. The transformed data were statistically evaluated by analysis of variance for randomized block designs, and comparisons of treatment means to those of saline controls were made by a two-tailed Dunnett's t test. A two-way analysis of variance was used to compare the dose-response curve for naloxone in unpretreated rats with the naloxone dose-response curves following pretreatment with morphine (Fig. 4). A p value of less than 0.05 was selected as the upper limit for statistical significance.

RESULTS

Suppression of Drinking by Naloxone and Naltrexone

The absolute volumes of water (by body wt) consumed by water-deprived homo- and heterozygous Brattleboro rats under control conditions of each drug series are presented in the legend of Fig. 1. Although there was little variability in baseline water intake within each genotype, 1 hr water-deprived homozygotes consumed more water under control conditions than did 24 hr deprived heterozygotes, a difference which was significant only between those groups tested with naloxone ($p<0.01$).

Both naloxone and naltrexone (0.01–10 mg/kg) decreased water consumption induced by deprivation in homo- and heterozygous Brattleboro rats (Fig. 1). The water intake of homozygotes was reduced by 19 and 27% by 1.0 mg/kg of

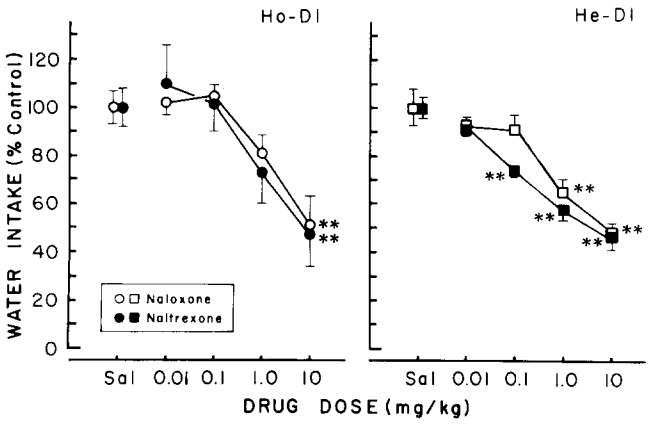

FIG. 1. Suppression of water intake by naloxone and naltrexone in Brattleboro rats homozygous (Ho-DI) and heterozygous (He-DI) for diabetes insipidus. Homo- and heterozygotes were deprived of water for 1 and 24 hr, respectively, prior to testing. Each point represents the mean±S.E. of 9 observations in homozygotes and 12 observations in heterozygotes. The absolute values of water intake obtained in tests with isotonic saline (points above Sal) are: 49.7±3.5 and 37.8±2.8 ml/kg of body weight for naloxone-treated homo- and heterozygotes, respectively, and 47.1±3.8 and 39.9±1.8 ml/kg of body weight for naltrexone-treated homo- and heterozygotes, respectively. Significant differences between control and treatment means are indicated as $^{**}p<0.01$.

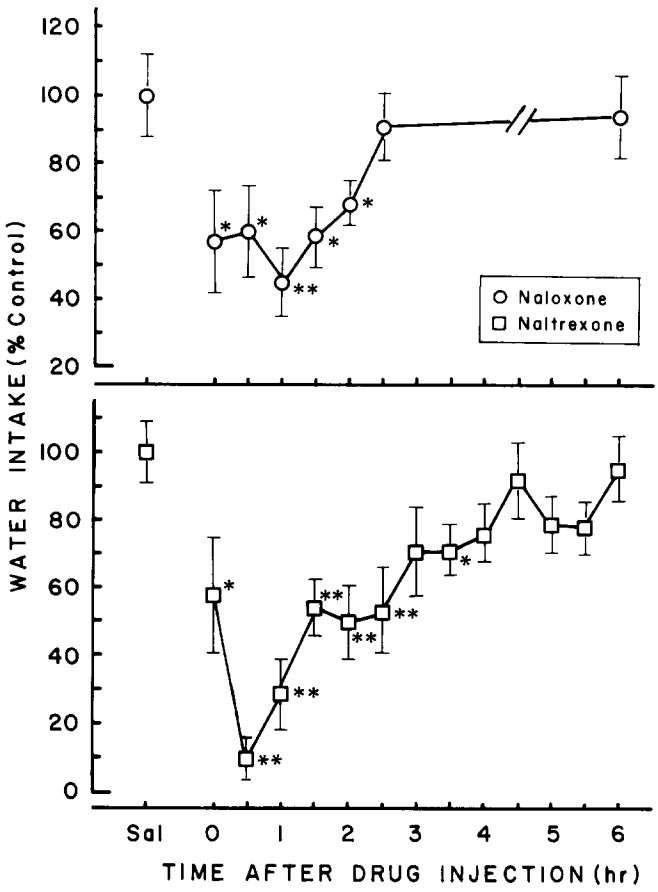

FIG. 2. Time course for the suppression of water inake by 3.0 mg/kg of naloxone (upper graph) and naltrexone (lower graph) in homozygous Brattleboro rats. Subjects were deprived of water for 1 hr prior to testing. Water intake measurements were conducted for 30 min following 0–6 hr drug pretreatment. Each point represents the mean±S.E. of 6–7 observations. The absolute values obtained in tests with isotonic saline (points above Sal) are 44.9±5.3 and 42.4±3.6 ml/kg of body weight for naloxone- and naltrexone-treated groups, respectively. Significant differences between saline control and treatment means are indicated as: $^{*}p<0.05$, and $^{**}p<0.01$.

naloxone and naltrexone, respectively. Ten mg/kg of naloxone and naltrexone further decreased water intake by 49 and 53%, respectively (Fig. 3). Naltrexone, in doses as low as 0.1 mg/kg, significantly decreased water consumption by 26% in heterozygotes. One mg/kg of naloxone and naltrexone reduced drinking by 35 and 42%, respectively (Fig. 3). The variability of the response of the heterozygotes to antagonists tended to be less than that of the homozygotes at each of the doses tested.

The duration of the suppressant effects of naloxone and naltrexone on drinking was determined in water-deprived homozygous Brattleboro rats. Each drug was administered at a dose of 3.0 mg/kg, 0–6 hr prior to testing. Both drugs produced a significant reduction in water intake which was rapid in onset, being apparent within 30 min after drug administration (Fig. 2). The drugs differed with respect to their peak suppressant effects: naltrexone maximally reduced water consumption by 90%, whereas naloxone decreased drinking by 55%. Furthermore, the suppressant effects of naltrexone were longer in duration than those of naloxone. A significant suppression of water intake remained up to 2.0 and 3.5 hr following the administration of naloxone and naltrexone, respectively (Fig. 2).

Effects of Naloxone on Drinking after Morphine Pretreatment

Water-deprived homozygotes pretreated with 10 mg/kg of morphine 3 hr prior to testing with isotonic saline exhibited no differences in water consumption compared to control conditions in which rats were pretreated with saline 3 hr prior to a second injection of saline (cf. legend, Fig. 3). However, morphine (0.1–17.5 mg/kg) administered 3 hr prior to testing enhanced the suppressant effects of 1.0 mg/kg of naloxone on drinking in a dose-related manner. One mg/kg of naloxone alone reduced water consumption by 32%, and pretreatment with 0.3–3.0 mg/kg of morphine further decreased water intake after naloxone administration by 40 to 60% (Fig. 3). Maximal enhancement of the suppressant actions of naloxone was apparent following 10 mg/kg of morphine; at this dose, water intake was diminished by 79%.

The effects of morphine on the ability of naloxone to attenuate drinking were time-dependent as well as dose-related. The optimum dose of morphine, 10 mg/kg, was administered 3–48 hr prior to testing the suppressant activity of 0.01–10 mg/kg of naloxone on drinking in water-deprived homozygotes. This dose of morphine had no effects on water

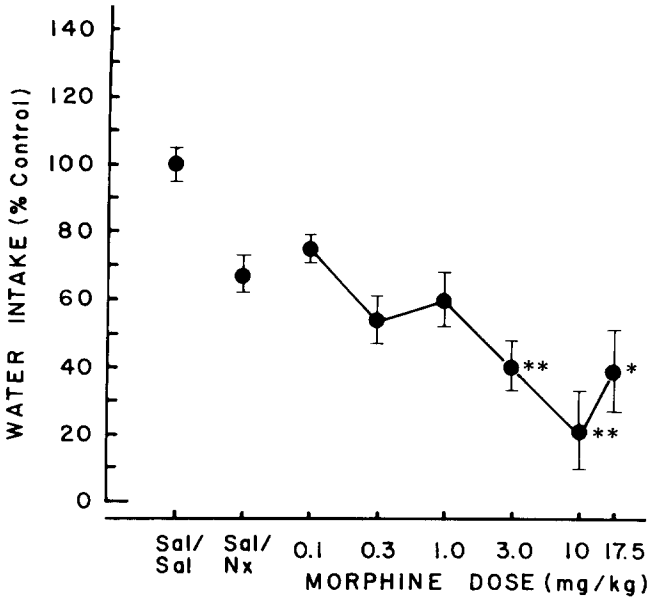

FIG. 3. Dose-dependent potentiation by morphine of the suppression of water intake by 1.0 mg/kg of naloxone in homozygous Brattleboro rats. Subjects were deprived of water for 1 hr prior to testing. Morphine (0.1–17.5 mg/kg) was administered 3 hr prior to water intake tests. Each point represents the mean±S.E. of 11 observations. The absolute volume of water consumption obtained in tests with isotonic saline (point above Sal-Sal) is 40.0±1.9 ml/kg of body weight. The absolute volume of water intake following the 3 hr pretreatment with 10 mg/kg of morphine in the absence of naloxone (not shown) was 50.0±5.1 ml/kg of body weight, which was not significantly different from the saline control value ($p>0.05$). Significant differences between naloxone suppressant activity in the absence (point above Sal-Nx) and presence of morphine are indicated as: *$p<0.05$, and **$p<0.01$.

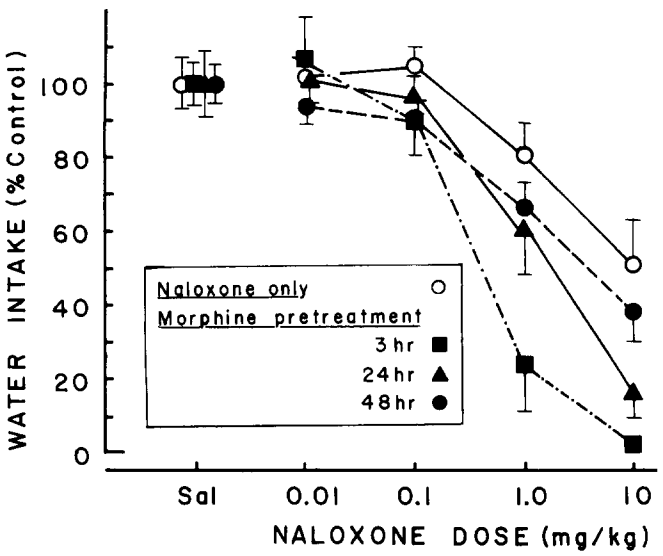

FIG. 4. Time-dependent effects of morphine (10 mg/kg) on the suppression of water intake by naloxone in homozygous Brattleboro rats. Subjects were deprived of water for 1 hr prior to testing. Morphine was administered 3, 12, 24, or 48 hr prior to water intake determinations. The naloxone dose-response after 12 hr morphine pretreatment was similar to the one obtained after 24 hr pretreatment and is omitted for purposes of clarity. The dose-response curve of naloxone in the absence of morphine (naloxone only) in homozygotes is the same one that appears in Fig. 1. Each point represents the mean±S.E. of 9 observations in all groups undergoing morphine pretreatment. The absolute values obtained in tests with isotonic saline are: 42.3±2.7, 60.1±5.3, and 54.1±2.9 ml/kg of body weight in the 3, 24, and 48 hr morphine-pretreated groups, respectively. All dose-response curves for naloxone after pretreatment with morphine are significantly different ($p<0.05$) from the dose-response curve for naloxone in the unpretreated rats.

intake determined 3–48 hr later. However, naloxone significantly reduced ($p<0.01$) water intake by 76 and 98% at doses of 1.0 and 10 mg/kg, respectively, following 3 hr pretreatment with morphine (Fig. 4). This enhancement of the effects of naloxone on drinking diminished with longer morphine pretreatment intervals. Thus, 1.0 and 10 mg/kg of naloxone decreased drinking by 40 and 84% in animals treated with morphine 24 hr previously (Fig. 4). The effects of 12 hr morphine pretreatment (not shown) were similar to those observed after 24 hr pretreatment. The dose-effect curve for naloxone after 48 hr morphine pretreatment was still significantly different ($p<0.05$) from that of naloxone in the absence of morphine (Fig. 4).

All drugs, at the doses tested, had no significant effects on either the overt behavior at the time of testing, or the general health of any experimental subject.

DISCUSSION

The narcotic antagonist, naloxone has previously been shown to suppress drinking in normal rats induced by a wide variety of dipsogenic stimuli. The results of this study extend these findings to drinking in rats with hereditary hypothalamic diabetes insipidus. Homozygous Brattleboro rats display a complete inability to synthesize or secrete vasopressin, and the copious water consumption of these animals, accentuated by mild water deprivation, was reduced by naloxone and its structural congener, naltrexone in a dose-related manner. Thus, vasopressin does not appear to be essential for the elaboration of the antidipsogenic effect of narcotic antagonists. Furthermore, previous studies have examined the effects of naloxone solely in Sprague-Dawley-derived rat strains. The present observations extend these findings to the Long Evans rat strain, to which the Brattleboro rat is related [39]. Indeed, the water intake of 24 hr water-deprived heterozygous Brattleboro rats, which possess low levels of vasopressin, was suppressed by naloxone in a dose-related pattern similar to that previously observed in normal, Sprague-Dawley-derived rats [5].

The onset of the antidipsogenic effects of both naloxone and naltrexone was apparent immediately after the administration of both drugs, probably due to their rapid tissue distribution [1]. However, naltrexone had a greater potency and longer duration of action in attenuating water consumption relative to naloxone. These results are consistent with the greater potency and duration of action of naltrexone compared to naloxone in other procedures involving drug inter-

actions with the opiate receptor, such as binding affinity [9], the precipitation of withdrawal-jumping in morphine-dependent mice [8] and the precipitation of an abstinence syndrome in morphine-dependent monkeys [42] and humans [26].

Morphine enhances the suppressant effects of naloxone on drinking in homozygous Brattleboro rats. The magnitude of this enhancement of naloxone activity is related to both the dose of morphine and the length of the morphine pretreatment interval. The morphine-induced potentiation of the antidipsogenic effects of naloxone was evident for at least 48 hr; this time interval is longer than that obtained in a previous study on the ability of morphine to increase the potency and efficacy of naloxone in antagonizing morphine-induced analgesia in mice [37]. However, the antidipsogenic activity of naloxone was not modified in postmorphine-dependent rats tested 7 days after the withdrawal of morphine [17].

The increased activity of naloxone after opiate agonist treatment may be the consequence of an agonist-induced alteration in the conformation of the opiate receptor, thereby increasing the affinity of narcotic antagonists for the receptor [21, 35, 37, 38, 43]. This hypothesis has recently been disputed [12]. An alternative explanation for these effects is that the administration of even a single dose of morphine engenders a state of acute dependence such that the observed decreases in water intake after naloxone treatment are actually a manifestation of a precipitated abstinence syndrome [33]. These possibilities cannot be resolved on the basis of the present data.

In summary, the reported abnormalities in the opiate and vasopressin systems of homozygous Brattleboro rats did not seem to modify the antidipsogenic effects of the narcotic antagonists. Both homozygotes and heterozygotes responded to drug administration in a manner similar to that observed in normal rats. Thus, vasopressin does not appear to mediate the actions of naloxone on water intake. Owing to the generality of the antidipsogenic effects of naloxone, it is conceivable that the drug is acting on an endogenous opioid pathway subserving water consumption. Indeed, sites of endorphinergic fiber investments in the hypothalamus [3] lie in close proximity to putative neural centers regulating water intake [20,27]. The endogenous opioids may also physiologically mediate vasopressin release from the pituitary [2, 18, 24]. Naloxone and other narcotic antagonists may act upon one component of an integrated endogenous opioid system involved in the maintenance of fluid homeostasis. The polydipsic Brattleboro rat appears to be a convenient animal model for further studies of the actions of opiate agonists and antagonists on drinking behavior.

REFERENCES

1. Berkowitz, B. A., S. Spector and C. H. Lee. Mechanism of narcotic antagonist and narcotic antagonist analgesic action. In; *Tissue Responses to Addictive Drugs*, edited by D. H. Ford and D. H. Clouet. New York: Spectrum Publications, 1976, pp. 139–153.
2. Bisset, G. W., H. S. Chowdrey and W. Feldberg. Release of vasopressin by enkephalin. *Br. J. Pharmac.* **62:** 370–371, 1978.
3. Bloom, F. E., E. Battenberg, J. Rossier, N. Ling and R. Guillemin. Neurons containing β-endorphin in rat brain exist separately from those containing enkephalin: immunocytochemical studies. *Proc. natn Acad. Sci. U.S.A.* **75:** 1591–1595, 1978.
4. Blumberg, H. and H. B. Dayton. Naloxone, naltrexone and related noroxymorphones. In: *Narcotic Antagonists*, edited by M. C. Braude, L. S. Harris, E. L. May, J. P. Smith and J. E. Villarreal. New York: Raven Press, 1974, pp. 33–43.
5. Brown, D. R. and S. G. Holtzman. Suppression of deprivation-induced food and water intake in rats and mice by naloxone. *Pharmac. Biochem. Behav.* **11:** 567–573, 1979.
6. Brown, D. R., M. S. Blank and S. G. Holtzman. Suppression by naloxone of water intake induced by deprivation and hypertonic saline in intact and hypophysectomized rats. *Life Sci.* **26:** 1535–1542, 1980.
7. Brown, D. R. and S. G. Holtzman. Suppression of drinking by naloxone in the rat: a further characterization. *Eur. J. Pharmac.* **69:** 331–340, 1981.
8. Cowan, A. Use of the mouse jumping test for estimating antagonistic potencies of morphine antagonists. *J. Pharm. Pharmac.* **28:** 177–182, 1976.
9. Creese, I. and S. H. Snyder. Receptor binding and pharmacological activity of opiates in the guinea-pig intestine. *J. Pharmac. exp. Ther.* **194:** 205–219, 1975.
10. Czech, D. A. and E. A. Stein. Naloxone depresses osmoregulatory drinking in rats. *Pharmac. Biochem. Behav.* **12:** 987–989, 1980.
11. DeWeid, D. and W. H. Gispen. Impaired development of tolerance to morphine analgesia in rats with hereditary diabetes insipidus. *Psychopharmacologia* **46:** 27–29, 1976.
12. Dum, J., J. Bläsig, G. Meyer and A. Herz. Opiate antagonist-receptor interaction unchanged by acute or chronic opiate treatment. *Eur. J. Pharmac.* **55:** 375–383, 1979.
13. Frenk, H. and G. H. Rogers. The suppressant effects of naloxone on food and water intake in the rat. *Behav. Neural Biol.* **26:** 23–40, 1979.
14. Glick, S. M. and M. J. Brownstein. Vasopressin content of rat brain. *Life Sci.* **27:** 1103–1110, 1980.
15. Goldstein, A. Endorphins: physiology and clinical implications. *Ann. N. Y. Acad. Sci.* **311:** 49–58, 1978.
16. Holtzman, S. G. Effects of narcotic antagonists on fluid intake in the rat. *Life Sci.* **16:** 1465–1470, 1975.
17. Holtzman, S. G. Suppression of appetitive behavior in the rat by naloxone: lack of effect of prior morphine dependence. *Life Sci.* **24:** 219–226, 1979.
18. Iversen, L. L., S. D. Iversen and F. E. Bloom. Opiate receptors influence vasopressin release from nerve terminals in rat neurohypophysis. *Nature* **284:** 350–351, 1980.
19. Jacob, J. Fonctions endocriniennes et neuroendocriniennes des morphines endogenès. *J. Physiol., Paris* **75:** 447–461, 1979.
20. Johnson, A. K. and J. Buggy. Periventricular preoptic-hypothalamus is vital for thirst and normal water economy. *Am. J. Physiol.* **234:** R122–R129, 1978.
21. Kitano, T. and A. E. Takemori. Further studies on the enhanced affinity of opioid receptors for naloxone in morphine-dependent mice. *J. Pharmac. exp. Ther.* **209:** 456–561, 1979.
22. Laycock, J. F. The Brattleboro rat with hereditary hypothalamic diabetes insipidus. *Gen. Pharmac.* **8:** 297–302, 1977.
23. Leander, J. D., D. E. McMillan and L. S. Harris. Effects of narcotic agonists and antagonists on schedule-induced water and morphine ingestion. *J. Pharmac. exp. Ther.* **195:** 271–278, 1975.
24. Lightman, S. L. and M. L. Forsling. Evidence for endogenous opioid control of vasopressin release in man. *J. clin. Endocr. Metab.* **50:** 569–571, 1980.
25. Maickel, R. P., M. C. Braude and J. E. Zabik. The effects of various narcotic agonists and antagonists on deprivation-induced fluid consumption. *Neuropharmacology* **16:** 863–866, 1977.
26. Martin, W. R., D. R. Jasinski and P. A. Mansky. Naltrexone, an antagonist for the treatment of heroin dependence: effects in man. *Archs gen. Psychiat.* **28:** 784–791, 1973.

27. Mogenson, G. J. and J. Kucharczyk. Central neural pathways for angiotensin-induced thirst. *Fedn Proc.* **37:** 2683–2688, 1978.
28. Möhring, B. and J. Möhring. Plasma ADH in normal Long-Evans rats and in Long-Evans rats heterozygous and homozygous for hypothalamic diabetes insipidus. *Life Sci.* **17:** 1307–1314, 1975.
29. Rigter, H., R. B. Messing, B. J. Vasquez, R. A. Jensen, J. L. Martinez, Jr., J. C. Crabbe, Jr. and J. L. McGaugh. Regional analysis of brain opiate receptors in rats with hereditary hypothalamic diabetes insipidus. *Life Sci.* **25:** 1137–1144, 1979.
30. Robertson, G. L. Vasopressin and water metabolism. In: *The Year in Endocrinology, 1977*, edited by S. H. Ingbar. New York: Plenum Press, 1978, pp. 205–231.
31. Rossier, J., Q. Pittman, F. Bloom and R. Guillemin. Distribution of opioid peptides in the pituitary: a new hypothalamic-pars nervosa enkephalinergic pathway. *Fedn Proc.* **39:** 2555–2560, 1980.
32. Sawynok, J., C. Pinsky and F. S. LaBella. On the specificity of naloxone as an opiate antagonist. *Life Sci.* **25:** 1621–1632, 1979.
33. Sparber, S. B., V. F. Gellert, L. Lichtblau and R. Eisenberg. The use of operant behavior methods to study aggression and effects of acute and chronic morphine administration in rats. In: *Factors Affecting the Action of Narcotics*, edited by M. L. Adler, L. Manara and R. Samanin. New York: Raven Press, 1978, pp. 63–91.
34. Stapleton, J. M., N. L. Ostrowski, V. J. Merriman, M. D. Lind and L. D. Reid. Naloxone reduces fluid consumption in water-deprived and nondeprived rats. *Bull. Psychonom. Soc.* **13:** 237–239, 1979.
35. Takemori, A. E., T. Oka and N. Nishiyama. Alteration of analgesic receptor-antagonist interaction induced by morphine. *J. Pharmac. exp. Ther.* **186:** 261–265, 1973.
36. Terenius, L. Endogenous peptides and analgesia. *A. Rev. Pharmac. Toxic.* **18:** 189–204, 1978.
37. Tulunay, F. C. and A. E. Takemori. The increased efficacy of narcotic antagonists induced by various narcotic analgesics. *J. Pharmac. exp. Ther.* **190:** 395–400, 1974.
38. Tulunay, F. C. and A. E. Takemori. Further studies on the alteration of analgesic receptor-antagonists interaction induced by morphine. *J. Pharmac. exp. Ther.* **190:** 401–407, 1974.
39. Valtin, H., H. A. Schroeder, K. Benirschke and H. W. Sokol. Familial hypothalamic diabetes insipidus in rats. *Nature* **196:** 1109–1110, 1962.
40. Valtin, H. and H. A. Schroeder. Familial hypothalamic diabetes insipidus in rats (Brattleboro strain). *Am. J. Physiol.* **206:** 425–430, 1964.
41. Valtin, H., W. H. Sawyer and H. W. Sokol. Neurohypophysial principles in rats homozygous and heterozygous for hypothalamic diabetes insipidus (Brattleboro strain). *Endocrinology* **77:** 701–706, 1965.
42. Villarreal, J. E. and M. G. Karbowski. The actions of narcotic antagonists in morphine-dependent rhesus monkeys. In: *Narcotic Antagonists*, edited by M. C. Braude, L. S. Harris, E. L. May, J. P. Smith and J. E. Villarreal. New York: Raven Press, 1974, pp. 273–289.
43. Wong, C. -L. and G. A. Bentley. Increased antagonist potency of naloxone caused by morphine pretreatment in mice. *Eur. J. Pharmac.* **47:** 415–422, 1978.