Pharmacology Biochemistry & Behavior, Vol. 16, pp. 87–91, 1982. Printed in the U.S.A.

Comparison of the Suppression by Naloxone of Water Intake Induced in Rats by Hyperosmolarity, Hypovolemia, and Angiotensin

NEIL ROWLAND[1]

Department of Psychology, University of Pittsburgh, Pittsburgh, PA 15260

Received 21 July 1981

ROWLAND, N. *Comparison of the suppression by naloxone of water intake induced in rats by hyperosmolarity, hypovolemia, and angiotensin.* PHARMAC. BIOCHEM. BEHAV. 16(1) 87–91, 1982.—The effect of naloxone upon water consumption by rats was assessed using two intensities each of IV NaCl (hyperosmolarity), SC polyethylene glycol (hypovolemia), and IV angiotensin II. In each case naloxone produced a dose-related reduction in the amount drunk. Angiotensin-induced drinking was most easily inhibited, and was abolished by only 1 mg/kg naloxone. In contrast, 1 mg/kg naloxone produced only a 50% reduction in NaCl-induced drinking, and hypovolemia-induced drinking was not completely reversed by 5 mg/kg. Naloxone was without effect upon the natriuresis after NaCl, or the hypertension during AII administrations. Parallels are drawn between the effects of naloxone on these types of thirst, and of other perturbations including brain damage and taste adulteration.

Water intake Opioids Naloxone Hyperosmolarity Hypovolemia Angiotensin II Natriuresis Hypertension

N-ALLYL-NOROXYMORPHONE (naloxone) partially attenuates the amount of water consumed by water-deprived mammals [6, 8, 9, 10, 12, 16, 24, 26]. It is currently believed that this suppression of behavior may be mediated via the blockade of opiate receptors by naloxone, and that endogenous opioids may somehow be involved in the organization of drinking behavior (see [24] for a review).

The physiological signals to drink after water deprivation arise from fluid losses in both the intracellular and extracellular compartments, and possibly from elevated plasma levels of the dipsogenic hormone angiotensin II (AII) (reviewed in [21]). It is feasible to experimentally produce any one of these three physiological signals, the preferred methods being intravenous (IV) administration of NaCl (osmotic stimulus), subcutaneous (SC) injection of polyethylene glycol (PEG; hypovolemic stimulus), or IV infusion of AII (hyperangiotensinemia) [21].

The water intake of fluid-deprived rats is typically reduced up to 50% by high doses of naloxone, and one interpretation of this incomplete blockade might be that only one of the physiological signals described above is susceptible to naloxone's effects. It is therefore necessary to examine the effects of naloxone on water intake induced by NaCl, PEG, or AII separately. Some recent studies have made a start in this analysis, but for various reasons these reports are incomplete. For example, the large suppressions by naloxone of water intake induced in mice or rats by hyperosmolarity [2, 6, 7, 17] were all obtained with painful SC or IP routes of administration. In certain paradigms, SC or IP administrations can nonspecifically obfuscate drinking responses to NaCl which are observed with IV infusions [5, 19, 20, 21]. Drinking in response to PEG or AII (both given SC) were strongly attenuated by naloxone [17], but in that initial study we used only one dose of each dipsogen and one high dose of naloxone, so were unable to compare the degree of inhibition with dose-response data for other dipsogens. Finally, while this manuscript was in preparation, Brown and Holtzman [3] reported that naloxone (1 mg/kg) produced a 50% attenuation of water intake induced in rats by isoproterenol or intracranial AII. However, neither of these stimuli produce a physiological state resembling either hypovolemia or peripheral hyperangiotensinemia (e.g., [29]), and the results do not bear directly upon the issue of opioid involvement in extracellular thirst.

The major aim of the present study is to build upon the incomplete data described in the previous paragraph by using appropriate dipsogens tested with several doses of naloxone. A second important parameter is the intensity of the dipsogenic stimulus, a factor which has not yet been studied for naloxone with these acute challenges. There is, however, reason to investigate this parameter since it has been reported that naloxone attenuated water intake by 57%

[1]Send reprint requests to present address: Department of Psychology, University of Florida, Gainesville, FL 32611.

in 12-hr fluid deprived rats, while the same dose of naloxone produced only 29% inhibition in 24-hr deprived rats [8]. However, the absolute reduction of water intake was 6 ml in both cases. Thus, at a given dose, naloxone may attenuate water intake by a fixed amount rather than by a fixed percentage (see also [17]). In the present work we have used two doses of each dipsogen. The lower dose was chosen to produce an intake of 3–4 ml in the test period; this intake corresponds to the largest spontaneous drafts in rats. The higher dose was chosen to produce an intake of 6–8 ml, close to that produced by 12 hr fluid deprivation. These intakes, matched across stimuli, are thus in a physiologically relevant range.

METHOD

Animals

Seventy-three adult male Sprague Dawley rats (Zivic Miller), weighing 250–400 g were used. They were housed individually with food pellets (Wayne) and tap water available at all times unless stated. Overhead lights were on from 0600–1800 hr, and all testing was performed near the middle of the day when spontaneous drinking is minimal.

Surgery

All surgical procedures were carried out on anesthetized (2.5 ml/kg Equithesin) animals. Some of the rats were fitted with indwelling jugular vein catheters, described elsewhere [15], for intravenous (IV) infusions. They were allowed 3 days postoperative recovery prior to the start of testing, and were housed in infusion cages [15] throughout. Other animals were implanted with both a jugular vein catheter and a PE10 catheter into the abdominal aorta for recording mean arterial blood pressure 2 days later.

General Test Procedure

On test days, naloxone hydrochloride (Endo Labs) dissolved in 0.9% NaCl carrier was injected subcutaneously (SC) at the designated dose in a volume of 1 ml/kg 15 min before the start of the drinking test. During the test, tap water was available in a graduated burette fitted with a metal sipping tube, and intakes were recorded to the nearest 0.1 ml at predetermined intervals.

Hyperosmolarity. Hyperosmolarity was produced by the IV infusion of hypertonic NaCl. The animals (N=23, run in several batches) were connected to a syringe and infusion pump (Harvard Instruments) via PE60 tubing immediately after receiving the naloxone injection. The IV infusion of 2.0 M NaCl was started 15 min later. The infusions were given for 1 or 2 hr (giving totals of 2 and 4 mEq Na infused, respectively), and water intake was recorded every 30 min for 3 hr. Food was absent throughout. Animals were tested only once on a given day, with 1–2 days between tests. Most of them were first studied without naloxone pretreatment, then after one or more doses of naloxone. No rat received more than three naloxone tests, and most received a second no-naloxone trial. The average intake was used in cases in which a given animal received a repeated treatment.

During the NaCl infusion experiments, 4 of the rats were housed and tested in metabolism cages for measurement of the excretion of the Na load. Urine was collected every hour for 4 hr during and after the infusion of 4 mEq/2 hr NaCl. On another day, the test was repeated after 1.0 mg/kg naloxone (SC). Water was available during these tests. The urine volume was noted, and its Na concentrations determined by a flame photometric method. Cumulative Na excreted was calculated from the volume and concentration.

Hypovolemia. Hypovolemia was produced by SC administration of hyperoncotic colloid PEG. Depletion of the vascular volume occurs over a period of hours, and drinking dose not emerge for several hours [28]. We thus employed a paradigm of delayed access to water [17] so that the temporal structure of this drinking test was comparable to those for the other dipsogens. Rats were lightly etherized and were injected SC, in the middle of the back, with 5 ml of 20% or 30% PEG (FW 20,000; w/v in 0.9% NaCl). They were returned immediately to their home cages from which food and water had been removed. Naloxone was injected (IP, to be remote from the site of the PEG injection and edema) 5 hr 45 min later, and after another 15 min water was presented. Water intake was recorded every 30 min for 2 hr. Each rat (N=18) received a total of 2 PEG tests, separated by 1 week. Six additional controls received SC NaCl (5 ml 0.9%) instead of PEG as the initial injection, a second injection of 0.9% NaCl (1 mg/kg IP), and were then given a drinking test using the same time schedule as for the PEG tests.

Hyperangiotensinemia. Hyperangiotensinemia was produced by a continuous IV infusion of AII (ileu^5 isomer, endogenous to rat; Sigma) at a rate of 64 or 256 ng/10 μl/min. The rats (N=34) were injected with naloxone 15 min before the start of the infusion. All rats were first screened with AII and no naloxone, and then with two or more doses of naloxone on different days; a few rats served in both NaCl and angiotensin experiments, but their data are in no way different from those with a less extensive experimental history. Food was present during the AII infusion, but was virtually never eaten. Water intakes were recorded every 10 min throughout the test, but for brevity only the 30 and 60 min values will be reported. These times represent, respectively, soon after initiation of drinking in the no naloxone condition, and the end of the test. No drinking occurred immediately after the infusion before the rats were disconnected from the infusion line.

The mean arterial blood pressure was recorded from the aorta catheter of the two additional freely-moving rats using a Statham pressure transducer and a Grass 7 polygraph. Pressure was recorded before and during 10 min successive IV infusions of AII at rates of 64 ng/10 μl/min and 256 ng/40 μl/min. The infusion was then stopped for 1 hr to allow blood pressure to return to baseline. Naloxone (5 mg/kg, SC) was then injected and, starting 15 min later, the infusion sequence was repeated. Blood pressure reached a maximum during the first 5 min of these infusions; thereafter it remained steady, or fell very slowly.

Statistical Treatment

All results were analyzed using one-way analysis of variance and t-tests. We have made no assumptions concerning the shape of dose-inhibition curves, and thus have not calculated ED50's by a mathematical procedure.

RESULTS

Intravenous NaCl

The amount of water consumed was related to the dose of NaCl infused (Fig. 1). Almost all of the intake occurred during the infusions, with very little after the infusion was stopped (note the minimal intakes in the 2nd hour of the 2

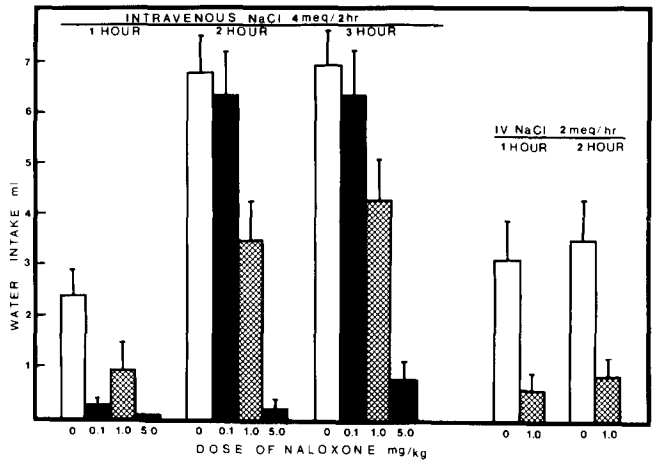

FIG. 1. Cumulative water intakes (M±SE) at various times during and after continuous intravenous (IV) infusion of 2 M NaCl in rats pre-injected (SC) with various doses of naloxone hydrochloride. Left: Infusion of 4 mEq NaCl at 2 mEq/hr (N's=22, 4, 17, 5 for the respective doses of naloxone shown). Right: Infusion of 2 mEq NaCl in 1 hr (N's=8). The intakes after 1 and 5 mg/kg naloxone are, in each case, significantly ($p<0.05$) less than the intake after no naloxone treatment (open bars).

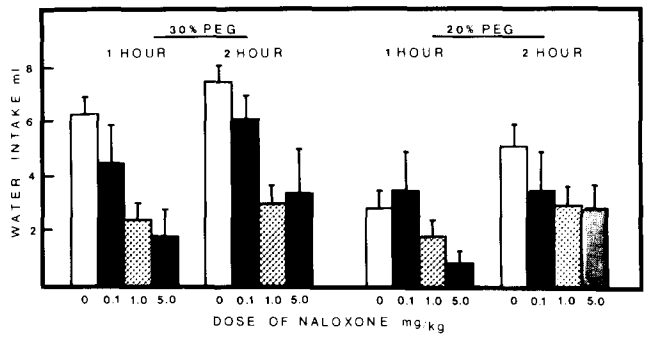

FIG. 2. Cumulative water intakes (M±SE) after 1 and 2 hr of a drinking test staring 6 hr after SC injection of polyethylene glycol (PEG). Rats were injected IP with vehicle (open bars) or various doses of naloxone hydrochloride 15 min prior to the drinking test. Left: Intakes after 1.0 (N=12), and 5.0 mg/kg (N=6) naloxone, but not after 0.1 mg/kg (N=6) were significantly ($p<0.05$) lower than the intakes of vehicle-treated rats (N=16) after 30% PEG. Right: Similarly for 20% PEG; N's=17, 6, 12, 5.

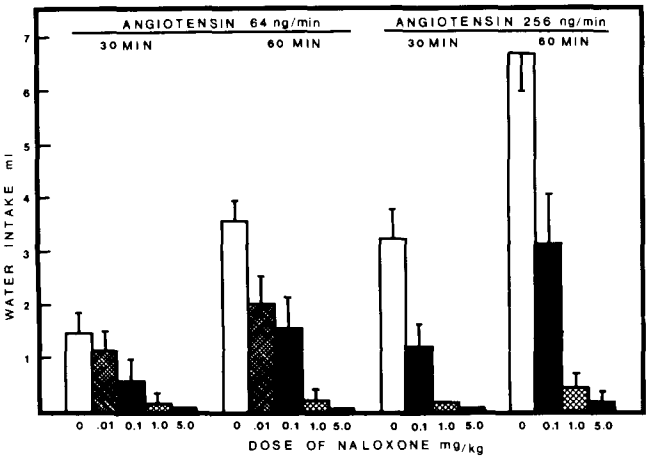

FIG. 3. Cumulative water intakes (M±SE) after 30 and 60 min of a continuous intravenous infusion of ^{5}ileu angiotensin II. Rats were injected SC with vehicle (open bars) or various doses of naloxone hydrochloride 15 min prior to the drinking test. In all cases, the intakes of animals treated with 0.1, 1.0 or 5.0 mg/kg naloxone were lower ($p<0.05$) than those of vehicle-treated rats, and the 0.01 mg/kg dose was effective ($p<0.05$) at 1 hr after 64 ng/min. N's (64 ng/min): 25, 11, 9, 9, 8; (256 ng/min): 22, 12, 11, 8.

mEq test and in the 3rd hour of the 4 mEq test). The drinking to 2 mEq NaCl was 81% attenuated by 1.0 mg/kg naloxone. In the animals which were infused with 4 mEq, at the end of the first hour (2 mEq infused) the intakes were 10% (0.1 mg/kg), 40% (1.0 mg/kg) and 0% (mg/kg) of control. At the end of the 2nd hour the respective percentage intakes were 93, 51 and 3. Notice that the overall suppression of intake expressed as percent was greater at the lower dose of NaCl (81%) than at the higher dose (49%: $p<0.05$). However, the reductions in absolute intake were not significantly different between the low (2.65 ml) and high (3.31 ml) doses of NaCl. Note also the apparent delay in response at 0.1 mg/kg.

The natriuretic response to NaCl infusion was not altered by naloxone (1 mg/kg) at any time during the test. In the first 4 hr after 4 mEq NaCl (infused during the first 2 hr) rats excreted 3.12±0.26 mEq Na (M±SE) in 13.4 ml urine, and drank substantial amounts (cf. Fig. 1). Following naloxone pretreatment, 3.30±0.26 mEq Na was excreted in 13.2 ml urine in 4 hr, despite much reduced water intake.

PEG-Induced Hypovolemia

Control PEG-treated rats drank throughout the 2 hr test, and for brevity only the intakes after 1 and 2 hr are shown in Fig. 2. Naloxone produced a dose-related suppression of PEG-induced drinking. The intakes, expressed as percent control, after 2 hr in the 30% PEG condition were: 0.1 mg/kg, 82%; 1.0 mg/kg, 40%; and 5 mg/kg, 46%. In the 20% PEG condition, the respective intakes were 68, 58 and 56%. The additional controls which did not receive PEG consumed no water during the 2 hr test. All of the observed intakes to PEG shown in Fig. 2 are thus above this zero baseline.

Intravenous AII

AII elicited water intake which increased both with duration of the test, and as a function of dose (Fig. 3). Following pretreatment with 1.0 mg/kg naloxone, water intake was reduced to less than 6% of control in all conditions (N's=9–11). The intakes after 0.1 mg/kg naloxone were 39% and 43% (64 ng/min at 30 min and 1 hr), and 37% and 39% (256 ng/min at 30 min and 1 hr) of control. An even lower dose (0.01 mg/kg naloxone) was effective in reducing water intake at the lower dose of AII (the higher dose was not tested).

Naloxone (5 mg/kg) altered neither basal nor AII-induced increments in mean arterial blood pressure. Only two animals completed this experiment, but their data were simi-

lar, quite clear, and negative. Before naloxone, the basal mean arterial pressure was 88.5 mm Hg, the steady level after 10 min 64 ng AII/min was 120.5 mm, and at 256 ng/min increased to 149.0 mm. The corresponding values after naloxone were 86.0, 124.5 and 152.0 mm Hg.

DISCUSSION

The present data confirm and extend previous work on the reduction of water intake by naloxone. Our results suggest that the efficacy of naloxone may differ among the various dipsogens.

The amount of water consumed during IV NaCl infusions was attenuated in a dose-related was by naloxone. Water intake was reduced by approximately 50% at a dose of 1.0 mg/kg naloxone, and was virtually abolished by 5.0 mg/kg. The percent decrease in intake was greatest for the 2 mEq NaCl, but the absolute reduction of intake was quite similar between the 2 and 4 mEq NaCl conditions. There was no consistent evidence that naloxone was increasing the latency to drink, or increasing the threshold, since little drinking occurred in the hour after the infusions. Further, there was no evidence that the physiological disposition of the infused NaCl differed between naloxone-treated and control groups, since the rate of Na excretion was identical in both groups. These data are quite comparable to the 70% reductions in water intake seen after single IP or SC injections of NaCl following 1.0 mg/kg naloxone [1, 6, 7, 17]. There is no reason, therefore, to regard those reductions as artifacts of the route of administration.

Water intake during hypovolemia (PEG) was likewise reduced by naloxone. As with the NaCl stimulus, PEG-induced intake was approximately 50% attenuated by 1.0 mg/kg naloxone. The hypovolemic stimulus differed from the osmotic stimulus insofar as higher doses of naloxone did not further attenuate PEG-induced water intake. However, Ostrowski *et al.* [17] observed 80% reduction in water intake of PEG-treated rats receiving a still higher dose of naloxone (10 mg/kg). In that study, saline-treated control rats had substantial baseline intakes, so the naloxone + PEG-treated rats were drinking that baseline and more. In the present work, the baseline was zero, so for a given number of ml reduction in intake, the percent reduction would be lower in the present study than in Ostrowski *et al.* [17]. The important fact is that a high dose of naloxone produced incomplete suppressions of water intake in both studies.

It should also be noted that the naloxone was administered IP to the PEG-treated rats in this study, which differs from our normal SC procedure. This finding may limit the certainty with which we can compare the various stimuli, since IP administered naloxone may be metabolized faster than after SC injection. In glucoprivic feeding experiments, the reduction in food intake lasted longer after SC injection [17]. There are additional measures which might have been taken to equate doses of naloxone but which were beyond the scope of this paper. These include a measurement of the rate of absorption and catabolism of naloxone in hypovolemia and/or direct IV administration of naloxone in all three stimulus situations.

The water intake induced by the IV infusion of AII was strongly inhibited by naloxone. A dose of 0.1 mg/kg reduced the intake by over 50%, and 1.0 mg/kg eliminated water intake. This reduction in water intake was not secondary to any alteration of the pressor response to AII which might, for example, have led to reduced penetration of the peptide hormone across capillary endothelial junctions in brain. The present infusions produce plasma levels of AII which only slightly exceed the normal physiological maximum [13], and so may be regarded as a more natural form of AII stimulus than either a SC bolus [17] or an intracerebroventricular injection [3]. In both of these latter cases the attenuation of AII-induced drinking by naloxone was either less complete [17] or naloxone was less potent [3] than in the present work, possibly because of the sudden intensity of the stimulus produced by the SC or intracerebroventricular routes. In unpublished experiments performed at the same time as those reported herein, 1.0 mg/kg naloxone produced only 50% reduction in water intake following 10 ng AII into the lateral cerebral ventricle. This result replicates the report of Brown and Holtzman [3] but also argues for the unphysiological nature of that stimulus. It may be concluded that, when the peptide AII is administered by the normal physiological route, the induced water intake is potently inhibited by naloxone. Whether this represents actions in brain or periphery, and at the same or different sites, is not addressed by the present data.

All of the present and published results of the effects of naloxone on water intake of animals given acute homeostatic challenges must be contrasted with those observed after water deprivation. Rats deprived of water for 24 hr show only a 30% reduction in drinking to doses as high as 10 mg/kg naloxone, with threshold doses of 0.5–1.0 mg/kg [6, 8, 9, 10, 16, 17, 26]. The reduction of water intake (in ml) was similar in 12 hr water deprived rats, although in terms of percent it was greater [8]. Comparing the deprivation results with those from the three dipsogens tested in this study suggests strong similarities between the PEG dipsogen and water deprivation, especially in dose-sensitivity to naloxone and magnitude of reduction.

These considerations have additional implications. First, AII cannot be the exclusive mediator of extracellular dehydration thirst, since a dose of naloxone which totally inhibited AII-induced drinking only slightly attenuated PEG-induced drinking. If we assume that naloxone does not interfere with the production of AII or renin during hypovolemia, then the contribution of AII to hypovolemic thirst must be quite small (see also [13,29]). Second, since isoproterenol-induced drinking was only partially inhibited by 1.0 mg/kg naloxone [3], a dose which completely abolished water intake induced by IV AII, then AII can at most be only a partial mediator of isoproterenol-induced drinking and a major contribution may come from hypotension per se (cf. [29]). Third, since water deprivation is thought to produce a combined intracellular and extracellular fluid deficit, then the naloxone inhibition data suggest that extracellular mechanisms predominate in the deprived rat. (If intracellular mechanisms were predominant then one would expect a more complete inhibition). This assertion is, however, directly refuted by an elegant physiological study [18] showing that extracellular depletion plays a minor role in deprivation thirst in rats. Clearly, other factors need to be considered.

We have argued that both initiation and termination of water consumption may be determined by fluid receptors both at peripheral and systemic levels [20, 21, 23]. In particular, environmental and taste factors have important facilitatory roles. In the latter case, we have shown that interference with normal taste leads to a disruption of regulatory water intake [20]. Of particular relevance are observations that adulteration of the drinking water with quinine

leads to substantial inhibition of drinking induced by acute stimuli such as those used in the present work, while deprivation-induced water intake is less affected [4, 14, 22]. Viewed from this perspective, our present data are consistent with an hypothesis that naloxone's effects upon water intake are mediated through an effect upon taste perception (water is less palatable). Such an hypothesis gains support from a recent demonstration that naloxone-treated rats show a diminished preference for sweet solutions, as if solutions tasted less sweet [11]. Le Magnen and his colleagues interpreted that result in terms of altered reinforcement mechanisms by naloxone. Such a position dovetails well with the implication of opioids in other types of motivated behavior and/or general reinforcement processes (e.g., [24, 25, 27]).

ACKNOWLEDGEMENTS

Work supported in part by NIH grant AM26231. I thank N. L. Ostrowski for helpful comments and J.-S. Yen for urine Na determinations. Thanks also go to Sharon Fink for typing the manuscript, and to an anonymous reviewer for substantially improving this communication.

REFERENCES

1. 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.
2. Brown, D. R. and S. G. Holtzman. Evidence that opiate receptors mediate suppression of hypertonic saline-induced drinking in the mouse by narcotic antagonists. *Life Sci.* **26:** 1543–1550, 1980.
3. 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.
4. Burke, G. H., D. G. Mook and E. M. Blass. Hyperreactivity to quinine associated with osmotic thirst in the rat. *J. comp. physiol. Psychol.* **78:** 32–39, 1972.
5. Coburn, P. C. and E. M. Stricker. Osmoregulatory thirst in rats after lateral preoptic lesions. *J. comp. physiol. Psychol.* **92:** 350–361, 1978.
6. Cooper, S. J. Naloxone: effects on food and water consumption in the non-deprived and deprived rat. *Psychpharmacology* **71:** 1–6, 1980.
7. Czech, D. A. and E. A. Stein. Naloxone suppresses osmoregulatory drinking in rats. *Pharmac. Biochem. Behav.* **12:** 987–989, 1980.
8. 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.
9. Holtzman, S. G. Behavioral effects of separate and combined administration of naloxone and d-amphetamine. *J. Pharmac. exp. Ther.* **189:** 51–60, 1974.
10. Holtzman, S. G. Suppression of appetitive behavior in the rat by naloxone: lack of prior morphine dependence. *Life Sci.* **24:** 219–226, 1979.
11. Le Magnen, J., P. Marfaing-Jallat, D. Miceli and M. Devos. Pain modulating and reward systems: A single brain mechanism? *Pharmac. Biochem. Behav.* **12:** 729–733, 1980.
12. 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.
13. Mann, J. F. E., A. K. Johnson and D. Ganten. Plasma angiotensin II: dipsogenic levels and angiotensin-generating capacity of renin. *Am. J. Physiol.* **238:** R372–R377, 1980.
14. Nicolaidis, S. and N. Rowland. Regulatory drinking in rats with permanent access to a bitter fluid source. *Physiol. Behav.* **14:** 819–824, 1975.
15. Nicolaidis, S., N. Rowland, M. J. Meile, P. Marfaing-Jallat and A. Pesez. A flexible technique for long term infusions in unrestrained rats. *Pharmac. Biochem. Behav.* **2:** 131–136, 1974.
16. Ostrowski, N. L., T. L. Foley, M. D. Lind and L. D. Reid. Naloxone reduces fluid intake: Effects of water and food deprivation. *Pharmac. Biochem. Behav.* **12:** 431–435, 1980.
17. Ostrowski, N. L., N. Rowland, T. L. Foley, J. L. Nelson and L. D. Reid. Morphine antagonists and consummatory behaviors. *Pharmac. Biochem. Behav.* **14:** 549–559, 1981.
18. Ramsay, D. J., B. J. Rolls and R. J. Wood. Body fluid changes which influence drinking in the water deprived rat. *J. Physiol.* **266:** 453–469, 1977.
19. Rowland, N. Regulatory drinking following lateral hypothalamic lesions: effects of chronic NaCl and water administrations. *Expl Neurol.* **53:** 488–507, 1976.
20. Rowland, N. Regulatory drinking: Do the physiological substrates have an ecological niche? *Biobehav. Rev.* **1:** 261–272, 1977.
21. Rowland, N. Drinking behaviour: Physiological, neurological and environmental factors. In: *Analysis of Motivational Processes*, edited by F. M. Toates and T. R. Halliday. London: Academic Press, 1980, pp. 39–59.
22. Rowland, N. and C. Flamm. Quinine drinking: More regulatory puzzles. *Physiol. Behav.* **18:** 1165–1170, 1977.
23. Rowland, N. and S. Nicolaidis. Metering of fluid intake and determinants of ad libitum drinking in rats. *Am. J. Physiol.* **231:** 1–8, 1976.
24. Sanger, D. J. Endophinergic mechanisms in the control of food and water intake. *Appetite* **2:** 193–208, 1981.
25. Stapleton, J. M., V. J. Merriman, C. L. Coogle, S. D. Gelbard and L. D. Reid. Naloxone reduces pressing for intracranial stimultion of sites in the periaqueductal gray area, accumbens nucleus, substantia nigra, and lateral hypothalamus. *Physiol. Psychol.* **7:** 427–436, 1979.
26. 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. Psychon. Soc.* **13:** 237–239, 1979.
27. Stein, L. and J. D. Belluzzi. Brain endorphins and the sense of well-being: A psychobiological hypothesis. In: *Advances in Biochemical Pharmacology*, vol. 18, edited by E. Costa and M. Trabucchi. New York: Raven Press, 1978, pp. 299–311.
28. Stricker, E. M. Some physiological and motivational properties of the hypovolemic stimulus for thirst. *Physiol. Behav.* **3:** 379–385, 1968.
29. Stricker, E. M. The renin-angiotensin system and thirst: some unanswered questions. *Fedn Proc.* **37:** 2704–2710, 1978.