*Pharmacology Biochemistry & Behavior*, Vol. 25, pp. 77–82, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

# Centrally-Administered Opioid Selective Agonists Inhibit Drinking in the Rat

ROBERT L. SPENCER,‡[1] DAVID DEUPREE,*SIGMUND HSIAO,*
HENRY I. MOSBERG,† VICTOR HRUBY,† THOMAS F. BURKS‡
AND FRANK PORRECA‡

*Department of *Psychology, †Chemistry and ‡Pharmacology, University of Arizona, Tucson, AZ 85721*

Received 19 November 1985

SPENCER, R. L., D. DEUPREE, S. HSIAO, H. I. MOSBERG, V. HRUBY, T. F. BURKS AND F. PORRECA. *Centrally-administered opioid selective agonists inhibit drinking in the rat.* PHARMACOL BIOCHEM BEHAV 25(1) 77–82, 1986.—The effects of intracerebroventricular injection of mu (morphine), kappa (dynorphin-(1–13), ethylketocyclazocine, and U50,488H), and delta ([D-Pen$^2$,D-Pen$^5$]enkephalin) opioid agonists on water intake of 14 hr water deprived rats was studied. All agonists caused a dose related decrease in time spent drinking, with a rank order potency of dynorphin-(1–13) > morphine > ethylketocyclazocine > [D-Pen$^2$,D-Pen$^5$]enkephalin = U50,488H. With the exception of morphine, all of the compounds increased the latency to begin drinking, but only at the highest doses tested. The rank order potency for this endpoint was dynorphin-(1–13) = ethylketocyclazocine > [D-Pen$^2$,D-Pen$^5$]enkephalin > U50,488H. The potent inhibition of drinking following centrally-given dynorphin-(1–13), at doses that did not affect the latency to begin drinking, supports a role for endogenous dynorphin in the homeostatic control of water balance. This function may not be primarily mediated through activation of a kappa opioid receptor since dynorphin-(1–13) was 80–230 times more potent than the selective kappa agonist, U50,488H or ethylketocyclazocine.

Opioids Drinking Water balance Dynorphin

FOLLOWING the discovery of opioid receptors and endogenous opioids within the central nervous system (CNS), a number of investigators have undertaken the task of delineating the contribution of opioids to the normal functioning of the organism. One of the many roles postulated for the opioids is the regulation of water intake. A variety of opioid antagonists has been shown to reduce deprivation-induced water consumption by rats following both systemic [3, 14, 22] and central [2,32] administration. Suppression of drinking by opioid antagonists has also been demonstrated in the golden hamster [21] and squirrel monkey [20]. Drinking in the rat, stimulated by isoproterenol [3] and angiotensin II (AII) [3,34], but not schedule-induced polydipsia [3], is also reduced following treatment with opioid antagonists. These effects appear to be centrally-mediated since they are seen with much lower doses of the opioid antagonists when given centrally [32]. Furthermore, systemic injections of quaternary antagonists (compounds that do not cross the blood brain barrier well) are ineffective [2].

The effects of agonists on water intake have not provided a consistent picture. Peripherally-given morphine increases cumulative water intake 1.25–4.5 hr after injection of a low dose (0.5–4 mg/kg) [14,22] but does not increase milk intake [20]. Water or milk consumption following higher doses of morphine are suppressed and a similar result is seen when lower doses are studied within 1 hr of administration [14, 20, 22]. Suppression of fluid intake may be due to the general depressant action of the opiate. Direct injection of morphine into the hypothalamus [5] or into the lateral cerebral ventricle [35] decreases drinking stimulated by AII or water deprivation.

Considerable evidence has accumulated suggesting the existence of multiple opioid receptor subtypes in mammals [1, 23, 24], raising the possibility that the previously reported inconsistent effects of opioids on fluid consumption could have resulted from differing actions at multiple opioid receptor types. Several studies have tested the effects on drinking of agonists and antagonists somewhat selective for the proposed subtypes of opioid receptors. No distinction was found between the potency of mu and delta preferring opioid antagonists on water intake [18]. Kappa preferring antagonists were found to be equipotent in reducing water consumption and antagonizing morphine analgesia, whereas mu preferring antagonists were much more potent in antagonizing morphine analgesia compared to their inhibition of drinking [19]. The proposed kappa agonist, ethylketocyclazocine, increases ad lib water [31] and sweetened milk intake [20] after systemic administration. Thus, a specific role of kappa opioid receptors in regulating the consumption of fluids has been suggested. This may be complementary to the increased urine production seen with kappa agonists, presumably resulting by inhibition of vasopressin release [17,33]. Taken together, these effects on water consumption and retention suggest a possible role for an endogenous kappa opioid in the regulation of water balance.

[1]Requests for reprints should be addressed to Robert L. Spencer, Department of Pharmacology, University of Arizona, Tucson, AZ 85724.

Dynorphin, an opioid peptide found in specific tracts throughout the rat brain [38], has been suggested to have high affinity for kappa receptors [6, 15, 27], and is thus a candidate regulator of water balance. Hypothalamic and neurointermediate pituitary levels of dynorphin have been found to vary with water deprivation [29]. The present study attempted to investigate further the role of endogenous opioids on the control of water intake by studying the intracerebroventricular (ICV) effects of the proposed kappa opioid agonists dynorphin, ethylketocyclazocine [39] and U50,488H [16,36]. The standard mu receptor opiate, morphine, and the novel proposed delta receptor agonist, [D-Pen$^2$, D-Pen$^5$]enkephalin (DPDPE) [7,25], were included for comparison. Both duration of time spent drinking and the latency to begin drinking were taken as endpoints in an effort to separate general depressant effects of the compounds from specific effects on water intake.

## METHOD

### *Subjects*

Subjects were 60 male, albino, Sprague-Dawley rats (150–200 g). They were housed in individual wire mesh cages, 20×18×25 cm (the wire mesh grids were 1×1 cm). The home room was maintained on a 12 hr light/dark cycle (lights on at 7:00 a.m.). Food (Purina Rat Chow) was available ad lib, except during the test sessions. Tap water was available except during experimental water deprivation.

### *Test Apparatus*

Animals were tested in individual cages, similar to their home cages. The test cages were suspended from a modified housing rack to which a pivoting metal arm was fixed. Attached to the metal arm were 25 ml burets fitted with a drinking spout on one end. The burets were positioned such that when the metal arm was lowered into place a glass buret was aligned in front of each of the test cages. During testing there was a 1 mm gap between the end of the drinking spouts and the front of each cage. Thus, each rat had to extend its tongue through the wire mesh cage in order to make contact with the drinking spout. This prevented contact with the spout, except during drinking. Each spout and test cage were part of an electric circuit with the rats serving as switches in that circuit. The circuit allowed less than 10 microamperes to pass through each animal, and rats showed no sign of aversion to licking with the current on, nor did the volumes of water consumed differ significantly between trials when current was on or off. The on-off state of the circuit was passed into a Grass polygraph recorder, with each lick producing a single pen deflection.

### *Cannulation*

Following induction of anesthesia with Innovar (0.05 ml, IM), a guide cannula (prefilled with silicone) was placed 2 mm posterior and 2 mm lateral on the left side to bregma. Two jewelers screws were also placed in the skull to help secure a dental acrylic mound built up around the guide cannula. Injections were made by inserting a 30 gauge 1/2 inch needle through the guide cannula. The needle was attached to a 10 μl Hamilton syringe and penetrated to a depth of 4 mm below the surface of the skull into the lateral ventricle. ICV injections were verified physiologically by observing drinking induced by ICV angiotensin I (500 ng) as well as evidence at necropsy of perfusion of an ICV injected methylene blue solution (5 μl) throughout the ventricular system.

### *Training and Testing Procedures*

Animals were given three training days in order to become familiar with the test cage and the location of the drinking spout. ICV cannulas were implanted and, following a 2-day recovery period, animals received one more day of training. The last training day included an ICV injection of sterile water. Testing then took place on the following four consecutive days. All training and test sessions occurred during the first third of the light cycle and after 14 hr of water deprivation. Each rat received four different doses of one of the test compounds over the four test days. The dose order was counterbalanced between rats. All injections were given 10 min prior to the start of each test session. Each test session lasted 30 min, during which time food was not available.

### *Drug Administration*

The test drugs were as follows: ethylketocyclazocine methanesulfonate (Sterling Winthrop), dynorphin A-(1–13) (Peninsula), U50,488H (Upjohn), morphine sulfate (Mallinckrodt), [D-Pen$^2$,D-Pen$^5$]enkephalin (synthesized as previously described [25]), and naloxone hydrochloride (Endo). All compounds, except dynorphin, were dissolved in distilled water. Dynorphin was dissolved in methyl alcohol/0.1 M hydrochloric acid, 1:1 v/v to prevent loss of the peptide through adsorption to the various glass and plastic surfaces which the compound comes in contact with during storage and delivery [12]. Drugs were injected, slowly by hand, ICV in 5 microliter volumes. Control injections utilized the appropriate vehicle.

### *Dependent Measures*

The chart recorder records were used to determine the amount of time each rat spent drinking during a test session, as well as the latency to begin drinking. Time spent drinking consisted of the sum duration of licking bursts, where a burst was defined as a series of licks with less then 0.5 sec between each successive lick. Measurement of the time spent drinking allowed evaluation of the amount of drinking at various time points throughout the test session without disturbing the subjects, which often occurs when approaching the test apparatus in order to take volumetric readings. Pilot studies found a 0.94 correlation between time spent drinking and amount consumed.

### *Data Analysis*

The results for each compound were analyzed separately. Repeated measure one-way analysis of variance tests were performed on the cumulative time spent drinking after 10, 20 and 30 min, and on the latency to begin drinking. Specific post-hoc comparisons were performed with the Newman-Keuls test of significance [26].

## RESULTS

All agonists tested caused a dose-related decrease in time spent drinking during the first 10 and 20 min of the test sessions (see Fig. 1 for sample records). The same compounds, except for ethylketocyclazocine, decreased time spent drinking for the total 30 min test period. Rats treated with ethyl-

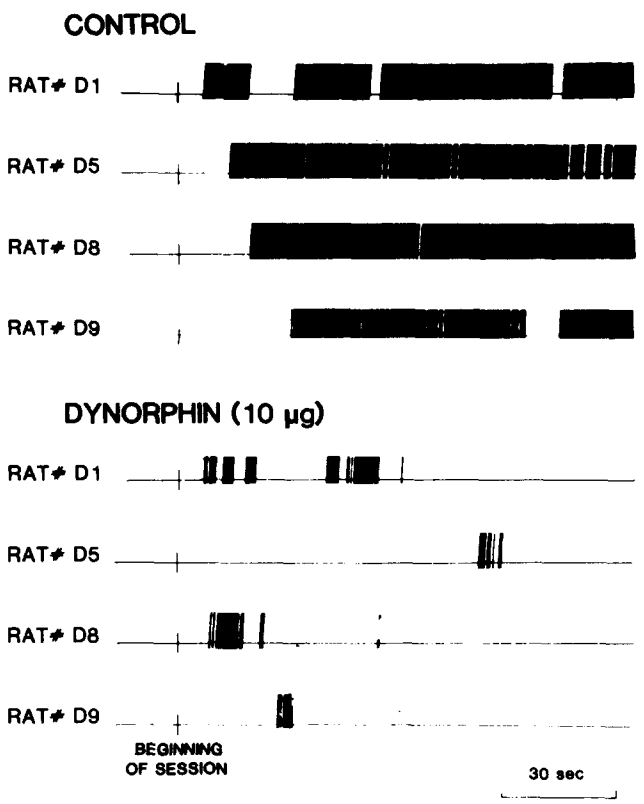


FIG. 1. Chart records of the first 2 min of drinking for 4 rats under conditions of control injection and dynorphin-(1–13) (10 g ICV). Note the greatly reduced amount of drinking with dynorphin-(1–13) treatment without significant increase in latency to begin drinking in these 4 rats.

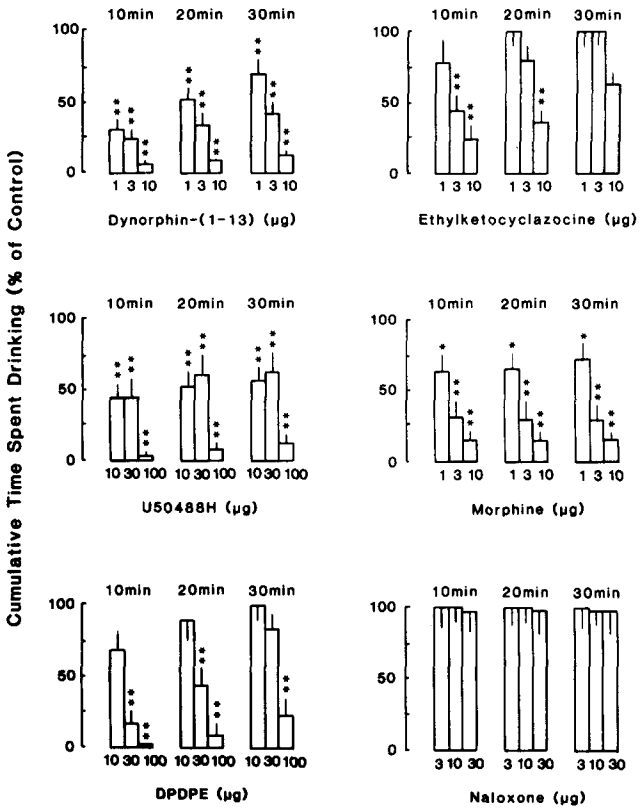


FIG. 2. Changes in cumulative time spent drinking expressed as a percent of vehicle control drinking time, at 10, 20 and 30 min after ICV injection of dynorphin-(1–13) (n=13), ethylketocyclazocine (n=11), U50488H (n=10), morphine (n=8), DPDPE (n=8) and naloxone (n=10). Neuman-Keuls test was used to determine significant differences between drug treatment and control conditions: *$p<0.05$ and **$p<0.01$.

TABLE 1

MEAN (±SEM) AMOUNT OF WATER CONSUMED (ml) IN 30 MINUTES

| | | Dose (µg) | | | | | |
|---|---|---|---|---|---|---|---|
| Drug | n | 0 | 1 | 3 | 10 | 30 | 100 |
| Dynorphin-(1–13) | 13 | 6.24(0.62) | 4.20(0.53)* | 2.58(0.56)* | 0.65(0.27)* | — | — |
| Ethylketocyclazocine | 11 | 8.27(0.82) | 7.75(0.64) | 6.84(0.63) | 5.18(1.20) | — | — |
| U50,488H | 10 | 7.27(0.60) | — | — | 4.08(0.94)* | 3.65(0.85)* | 1.51(0.82)* |
| Morphine | 8 | 6.92(0.30) | 4.24(0.82)* | 1.84(0.60)* | 0.76(0.40)* | — | — |
| DPDPE | 8 | 5.12(0.46) | — | — | 5.30(0.48) | 4.09(0.38) | 0.88(0.51)* |
| Naloxone | 10 | 8.45(0.75) | — | 8.42(0.46) | 7.53(0.59) | 7.06(0.57) | — |

*Significantly different (Neuman-Keuls test) from control condition, $p<0.01$.

ketocyclazocine increased drinking during the last 10 min so that the drug effect was not statistically significant for the total session. The dose- and time-related effects of each drug on drinking is presented in Fig. 2. The total volume of water consumed during the control condition for each drug ranged from a mean (±SEM) of 5.12 (0.46) ml, for the DPDPE control condition, to 8.45 (0.75) ml, for the naloxone control condition (see Table 1). The mean amount of water consumed for the DPDPE control condition was significantly less (Neuman-Keuls test) than for the naloxone and ethylketocyclazocine control conditions. None of the other means for the control conditions were significantly different from one another. The $D_{50}$ for each drug (the dose (nmol) which decreased drinking time by 50%) is presented in Table 2. The rank order potency of the compounds for the first 10 and 20 min, based on the $D_{50}$ values, was dynorphin-(1–13) > morphine > ethylketocyclazocine > DPDPE = U50,488H (see Fig. 3). At the end of 30 min the relative potencies of ethyl-

TABLE 2

DOSES (nmol) OF OPIOID AGONISTS WHICH DECREASED AMOUNT OF TIME SPENT DRINKING BY 50%, AFTER 10 MIN, 20 MIN AND 30 MIN OF THE TEST SESSION

| Drug | $D_{50}$ (95% Confidence Interval) 10 min | 20 min | 30 min |
|---|---|---|---|
| Dynorphin-(1–13) | 0.10 (0.01– 1.13) | 0.72 (0.41– 1.24) | 1.38 (0.94– 2.01) |
| Morphine | 2.55 (1.29– 5.03) | 2.48 (1.24– 4.97) | 2.98 (1.76– 5.07) |
| Ethylketocyclazocine | 8.03 (4.31–14.97) | 18.16(10.31–31.97) | 51.86(16.01–167.96) |
| U50,488H | 23.27 (9.05–59.83) | 39.85(17.82–89.15) | 48.92(23.05–103.82) |
| DPDPE | 22.40(12.81–39.17) | 44.14(29.22–66.66) | 82.22(53.15–127.20) |

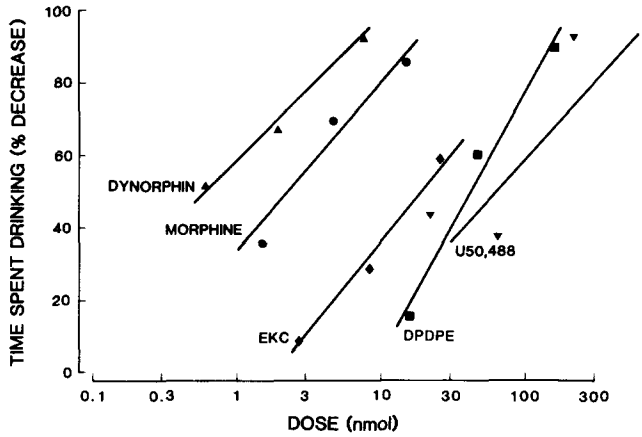


FIG. 3. Dose-response comparisons of the percent decrease in time spent drinking during the first 20 min after injection. Lines were drawn by regression analysis.

ketocyclazocine, U50,488H and DPDPE were essentially equal. Two rats receiving the highest dose of dynorphin-(1–13) (10 $\mu$g) displayed barrel rotations shortly after the injection. Although this behavior had ended before the beginning of the test session, their time spent drinking during the session was negligible. This phenomenon of short lasting barrel rotations after ICV dynorphin has been previously reported [13, 37, 40]. The doses of naloxone used in this study (3–30 $\mu$g, ICV) had no effect on water intake, and is consistent with other findings of ICV doses of naloxone below 50 $\mu$g [9,32].

Dynorphin, ethylketocyclazocine, U50,488H and DPDPE significantly increased the latency to begin drinking (see Fig. 4). Newman-Keuls post hoc analysis indicated that only the highest dose of each of the compounds, except DPDPE, was responsible for the increased latency. Both the intermediate and highest dose of DPDPE increased the latency to drink. The rank order of potency for this effect, based on the lowest effective dose for each drug, was dynorphin-(1–13) = ethylketocyclazocine > DPDPE > U50,488H. Interestingly, morphine, which decreased the time spent drinking, had no effect on latency to begin drinking.

## DISCUSSION

The present work has demonstrated a dose-related inhibition of drinking following the ICV administration of opioid agonists. These compounds were found to decrease the amount of time spent drinking. Additionally, the compounds increased the latency to begin drinking only at the highest doses tested. Interestingly, while morphine decreased time spent drinking, this compound did not affect latency to onset of drinking. The rank order potency of these effects was not identical, with dynorphin-(1–13) and morphine being most potent in reducing the time spent drinking, while morphine had no effect on latency to begin drinking. It is possible that the dynorphin-(1–13) vehicle, containing 50% methanol and 0.05 M HCl, altered the dispositional properties of dynorphin-(1–13), relative to the other compounds tested, thus partially accounting for the high potency of dynorphin-(1–13). Although this possibility cannot be ruled out, it seems unlikely given the comparable time course of action seen between all the compounds tested (see Fig. 2).

Though some other studies have observed an increase in drinking following treatment with opioid agonists [4, 14, 20, 22, 31], our experiments showed the reverse effect. It should be noted that most of those studies used peripheral administration of the compounds and that the effect was not evident for at least 1 hr after drug administration. One study reported increased drinking after ICV administration of [D-Ala$^2$, D-Leu$^5$]enkephalin (DADLE), but again did not see any effect until 1 hr after administration [4]. Those investigators concluded that the increase in drinking was an indirect effect caused by opioid-stimulated release of renin and subsequent AII formation. Other studies have reported decreases in drinking following central injections of morphine [5, 8, 35] as well as for the opioids, beta-endorphin [35], Met-enkephalin [10,35], Leu-enkephalin [10,35], and DADLE [8,10]. Thus, the initial and probably direct effect on the central nervous system of opioid related compounds is to decrease the amount of time spent drinking at low doses and to increase the latency to begin drinking at higher doses. Whether or not these effects involve modification of central motivation to drink (i.e. thirst) or reflect general activation, depressant or motor effects which interefere with drinking is difficult to determine. Nevertheless, the potent inhibition of drinking seen with dynorphin, at doses which did not increase the latency to drink, may indicate a role for dynorphin in water balance. Immunoreactive levels of dynorphin in rat hypothalamus and the intermediate lobe of the pituitary were found to vary with time of day and water deprivation [29]. During the daytime, dynorphin levels were low in the hypothalamus and high in the pituitary compared to nighttime levels. Water deprivation increased daytime levels of dynorphin in the hypothalamus, with a reciprocal reduction in the pituitary. Increased urination in the rat following injection of

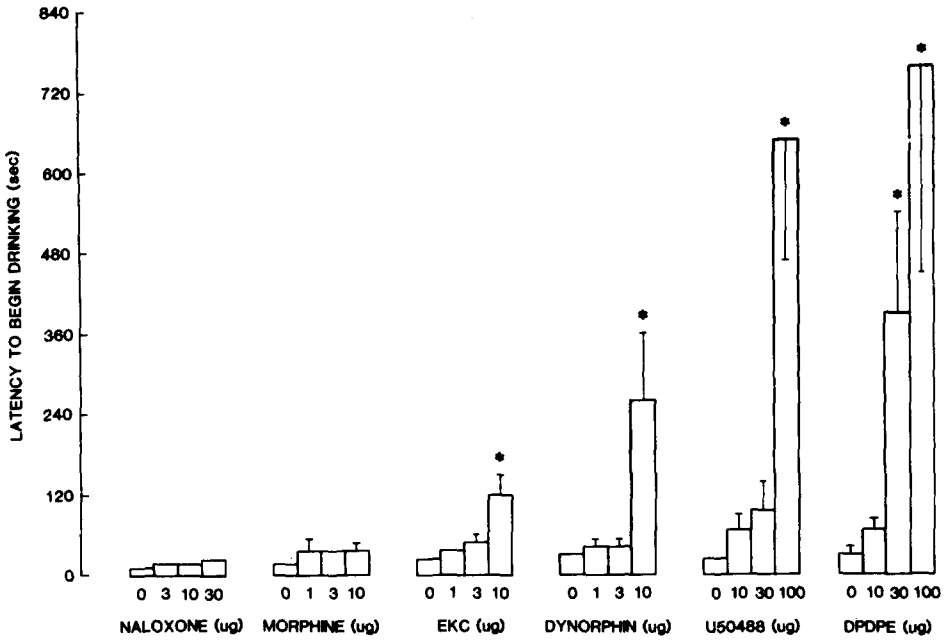


FIG. 4. Latency to begin drinking after injection with one of the opioid agonists. Neuman-Keuls test was used to determine significant differences between drug treatment and control conditions: $*p<0.01$.

kappa opioid agonists has also been reported [17,33]. The possibility of dynorphin inhibiting the release of antidiuretic hormone was suggested [17]. Inhibition by dynorphin of drinking together with inhibition of antidiuretic hormone release would provide for a coordinated regulation of water balance in the rat.

The two dependent measures, time spent drinking and latency to begin drinking, may reflect separate actions of the opioid agonists. Support for this comes from the fact that morphine and low doses of the other compounds decreased the amount of time spent drinking without affecting the latency to begin drinking. These separate actions may also show receptor subtype selectivity. The potent inhibition of drinking seen with dynorphin and morphine during the first 10 min of the test session, compared to the kappa and delta selective compounds, suggests that this may involve activation of an opioid receptor subtype other than the kappa or delta receptor. In the present study dynorphin-(1–13) was 80 and 230 times more potent than the kappa selective compounds, ethylketocyclazocine and U50,488H, respectively, and 220 times more potent than the delta compound, DPDPE. In the guinea-pig ileum bioassay, dynorphin-(1–13) is equipotent with ethylketocyclazocine [6,27]. For the same assay, dynorphin-(1–13) is 6–36 times more potent than U50,488H, when comparing values obtained from different laboratories [6, 27, 28]. Dynorphin and U50,488H are equipotent in displacing [$^3$H]ethylketocyclazocine in rat brain pretreated with dihydromorphine [16], or in bovine adrenal membrane pretreated with [D-Ala$^2$,Me-Phe$^4$,Gly$^5$-ol]-enkephalin [11]. They are also equipotent in producing analgesia in the mouse tail flick test, when administered intrathecally [28], and are equally potent at inhibiting acetylcholine evoked catecholamine secretion from isolated chromaffin cells [11]. Although dynorphin is generally considered to be a selective kappa agonist [6, 15, 27], some studies show considerable mu activity for dynorphin [16,30]. There is thus some possibility that the inhibition of drinking after ICV dynorphin was mediated through a mu receptor.

The increased latency to drink, however, may not involve mu receptor activation since morphine was ineffective in doses that reduced the time spent drinking. Another study, testing for AII stimulated drinking, also failed to see an effect of ICV morphine on the latency to begin drinking, although the volume of water consumed was reduced [35]. The increased latency to drink may be a result of kappa or delta receptor activation. Ethylketocyclazocine was nearly as potent as dynorphin-(1–13) at increasing the latency to begin drinking. The doses of DPDPE which decreased the time spent drinking also increased the latency to drink. DPDPE was the only compound tested which did not show a separation between doses which decreased the time spent drinking and doses which increased the latency to begin drinking. Thus, the effect of DPDPE on drinking may have been a unitary effect, primarily affecting latency which was also elicited with the high doses of dynorphin-(1–13), ethylketocyclazocine and U50,488H.

In conclusion, the potent inhibition of time spent drinking with dynorphin may be indicative of a role for the endogenous peptide in water balance regulation. This effect may not be a result of kappa or delta receptor activation since dynorphin-(1–13) was 80–230 times more potent than the selective kappa and delta compounds tested.

ACKNOWLEDGEMENTS

This work was supported by USPHS Grants AM 36289 (T.F.B.), DA02163 (T.F.B.) and NS21193 (F.P.).

## REFERENCES


1. Adler, M. W. Multiple opiate receptors and their different ligand profiles. Ann NY Acad Sci **398:** 340–351, 1982.
2. Brown, D. R. and S. G. Holtzman. Opiate antagonists: central sites of action in suppressing water intake of the rat. *Brain Res* **221:** 432–436, 1981.
3. Brown, D. R. and S. G. Holtzman. Suppression of drinking by naloxone in the rat: a further characterization. *Eur J Pharmacol* **69:** 331–340, 1981.
4. Cantalamessa, F., G. de Caro, M. Massi and L. G. Micossi. Stimulation of drinking behavior and of renin release induced by intracerebroventricular injection of D-ala$^2$,D-leu$^5$-enkephalin to rats. *Pharmacol Res Commun* **14:** 141–152, 1982.
5. Chance, W. T. and J. A. Rosecrans. Inhibition of drinking by intrahypothalamic administration of morphine. *Nature* **270:** 167–168, 1977.
6. Chavkin, C., I. F. James and A. Goldstein. Dynorphin is a specific endogenous ligand of the $\kappa$ opioid receptor. *Science* **215:** 413–415, 1982.
7. Corbett, A. D., M. G. C. Gillan, H. W. Kosterlitz, A. T. McKnight, S. J. Patterson and L. E. Robson. Selectivities of opioid peptide analogues as agonists and antagonists at the $\delta$-receptor. *Br J Pharmacol* **83:** 271–279, 1984.
8. Czech, D. A., M. J. Blake and E. A. Stein. Drinking behavior is modulated by CNS administration of opioids in the rat. *Appetite* **5:** 15–24, 1984.
9. Czech, D. A., E. A. Stein and M. J. Blake. Naloxone-induced hypodipsia: A CNS mapping study. *Life Sci* **33:** 797–803, 1983.
10. de Caro, G., L. G. Micossi and F. Venturi. Drinking behavior induced by intracerebroventricular administration of enkephalins to rats. *Nature* **277:** 51–53, 1979.
11. Dumont, M. and S. Lemaire. Interaction of dynorphin with kappa opioid receptors in bovine adrenal medulla. *Neuropeptides* **6:** 321–329, 1985.
12. Goldstein, A., S. Tachibana, L. I. Lowney, M. Hunkapiller and L. Hood. Dynorphin-(1–13), an extraordinarily potent opioid peptide. *Proc Natl Acad Sci USA* **76:** 6666–6670, 1979.
13. Herman, B. H., F. Leslie and A. Goldstein. Behavioral effects and in vivo degradation of intraventricularly administered dynorphin-(1–13) and D-ala$^2$-dynorphin-(1–11) in rats. *Life Sci* **27:** 883–892, 1980.
14. Jalowiec, J. E., J. Panksepp, A. J. Zolovick, N. Najam and B. H. Herman. Opioid modulation of ingestive behavior. *Pharmacol Biochem Behav* **15:** 477–484, 1981.
15. James, I. F., C. Chavkin and A. Goldstein. Selectivity of dynorphin for $\kappa$ opioid receptors. *Life Sci* **31:** 1331–1334, 1982.
16. Lahti, R. A., P. F. VonVoigtlander and C. Barsuhn. Properties of a selective kappa agonist, U-50,488H. *Life Sci* **31:** 2257–2260, 1982.
17. Leander, J. D. A kappa opioid effect: increased urination in the rat. *J Pharmacol Exp Ther* **224:** 89–94, 1983.
18. Leander, J. D., J. C. Hart, M. A. Lochner, M. D. Hynes and D. M. Zimmerman. Novel phenylpiperidine opioid antagonists and partial agonists: effects on fluid consumption. *Eur J Pharmacol* **81:** 185–192, 1982.
19. Leander, J. D. and M. D. Hynes. Opioid antagonists and drinking: evidence of $\kappa$-receptor involvement. *Eur J Pharmacol* **87:** 481–484, 1983.
20. Locke, K. W., D. R. Brown and S. G. Holtzman. Effects of opiate antagonists and putative mu- and kappa-agonists on milk intake in rat and squirrel monkey. *Pharmacol Biochem Behav* **17:** 1275–1279, 1982.
21. Lowy, M. T. and G. K. W. Yim. Drinking, but not feeding, is opiate-sensitive in hamsters. *Life Sci* **30:** 1639–1644, 1982.
22. 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.
23. Martin, W. R., C. G. Eades, J. A. Thompson, R. E. Huppler and P. E. Gilbert. The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. *J Pharmacol Exp Ther* **197:** 517–532, 1976.
24. Miller, R. J. Multiple opiate receptors for multiple opioid peptides. *Med Biol* **60:** 1–6, 1982.
25. Mosberg, H. I., R. Hurst, V. J. Hruby, K. Gee, H. I. Yamamura, J. J. Galligan and T. F. Burks. Bis-penicillamine enkephalins possess highly improved specificity toward $\delta$ opioid receptors. *Proc Natl Acad Sci USA* **80:** 5871–5874, 1983.
26. Myers, J. L. *Fundamentals of Experimental Design*, 3rd edition. Boston: Allyn and Bacon, Inc., 1979.
27. Oka, T., K. Negishi, M. Suda, A. Sawa, M. Fujino and M. Wakimasu. Evidence that dynorphin-(1–13) acts as an agonist on opioid kappa-receptors. *Eur J Pharmacol* **77:** 137–141, 1982.
28. Piercey, M. F., R. A. Lahti, L. A. Schroeder, F. J. Einspahr and C. Barsuhn. U-50488H, a pure kappa receptor agonist with spinal analgesic loci in the mouse. *Life Sci* **31:** 1197–1200, 1982.
29. Przewlocki, R., W. Lason, A. M. Konecka, C. Gramsch, A. Herz and L. D. Reid. The opioid peptide dynorphin, circadian rhythms, and starvation. *Science* **219:** 71–73, 1983.
30. Quirion, R. and C. Pert. Dynorphins: Similar relative potencies on $\mu$-, $\delta$- and $\kappa$-opiate receptors. *Eur J Pharmacol* **76:** 467–468, 1981.
31. Sanger, D. J. and P. S. McCarthy. Increased food and water intake produced in rats by opiate receptor agonists. *Psychopharmacology (Berlin)* **74:** 217–220, 1981.
32. Siviy, S. M., F. Bermudez-Rattoni, G. A. Rockwood, C. M. Dargie and L. D. Reid. Intracerebral administration of naloxone and drinking in water-deprived rats. *Pharmacol Biochem Behav* **15:** 257–262, 1981.
33. Slizgi, G. R. and J. H. Ludens. Studies on the nature and mechanism of the diuretic activity of the opioid analgesic ethylketocyclazocine. *J Pharmacol Exp Ther* **220:** 585–591, 1982.
34. Summy-Long, J. Y., L. C. Keil, K. Deen and W. B. Severs. Opiate regulation of angiotensin-induced drinking and vasopressin release. *J Pharmacol Exp Ther* **217:** 630–637, 1981.
35. Summy-Long, J. Y., L. C. Keil, K. Deen, L. Rosella and W. B. Severs. Endogenous opioid peptide inhibition of the central actions of angiotensin. *J Pharmacol Exp Ther* **217:** 619–629, 1981.
36. VonVoigtlander, P. F., R. A. Lahti and J. H. Ludens. U-50,488: a selective and structurally novel non-mu (kappa) opioid agonist. *J Pharmacol Exp Ther* **224:** 7–12, 1983.
37. Walker, J. M., H. C. Moises, D. H. Coy, G. Baldrighi and H. Akil. Nonopiate effects of dynorphin and des-tyr-dynorphin. *Science* **218:** 1136–1138, 1982.
38. Watson, S. J., H. Khachaturian, H. Akil, D. H. Coy and A. Goldstein. Comparison of the distribution of dynorphin systems and enkephalin systems in brain. *Science* **218:** 1134–1136, 1982.
39. Wood, P. L. and S. Charleson. High affinity [$^3$H]ethylketocyclazocine binding: evidence for specific $\kappa$ receptors. *Neuropharmacology* **21:** 215–219, 1982.
40. Zwiers, H., V. J. Aloyo and W. H. Gispen. Behavioral and neurochemical effects of the new opioid peptide dynorphin-(1–13): comparison with other neuropeptides. *Life Sci* **28:** 2545–2551, 1981.