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ANTIDIURETIC EFFECT OF β-ENDORPHIN AND MORPHINE IN BRATTLEBORO RATS: DEVELOPMENT OF TOLERANCE AND PHYSICAL DEPENDENCE AFTER CHRONIC MORPHINE TREATMENT

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Dedicated to Professor F. Huidobro on the occasion of his Fiftieth Anniversary as a teacher in Physiology and Pharmacology at the Catholic University, Santiago, Chile

1 β-Endorphin (2 μg injected into the lateral ventricles) produced a significant decrease in the urine outflow and in the excretion of Na^+ and K^+ in Brattleboro rats, animals suffering from severe diabetes insipidus. Morphine intracerebrally also produced antidiuresis, as compared to saline-treated controls.

2 Morphine injected intraperitoneally caused a dose-dependent decrease in the urine outflow, and in the excretion of Na^+ and K^+.

3 Rats chronically treated with morphine (72 h of morphine pellet implantation) were less sensitive to the antidiuretic effect of a challenge dose of morphine than control Brattleboro rats implanted with placebo pellets, but otherwise treated similarly.

4 After chronic morphine administration, Brattleboro rats became dependent on morphine. Challenge with 1 mg/kg naloxone (s.c.) precipitated an abrupt opiate withdrawal syndrome characterized, among other symptoms, by increased urination in contrast to the antidiuresis observed before naloxone.

Introduction

The mechanism of action of opiate-induced antidiuresis is not clearly understood. Despite the hypothesis proposed by De Bodo (1944) that opiates release antidiuretic hormone from the hypophysis/hypothalamus, little progress has been achieved in the past 35 years (Fujimoto, 1971). Recent evidence has shown that the profile of urine electrolyte excretion following the administration of synthetic vasopressin is markedly different and opposite from that obtained following the injection of morphine and other narcotic analgesics (Huidobro & Huidobro-Toro, 1979; Huidobro-Toro, Huidobro & Croxatto, 1979). These data coupled with the fact that morphine and related opiates injected peripherally or centrally produce a fall in peripheral blood pressure (Handley & Keller, 1950; Baker & Woods, 1957; Mills & Wang, 1964; Huidobro & Huidobro, 1970; Feldberg & Wei, 1977) and reduce the clearance of endogenous creatinine (Huidobro, Croxatto & Huidobro-Toro, 1979) cast doubts on whether the release of antidiuretic hormone is fully responsible for the morphine antidiuresis.

The purpose of this investigation was to evaluate whether the opioid-like peptide, β-endorphin and morphine produce antidiuresis and a reduction of the urine Na^+ and K^+ in Brattleboro rats. These animals lack antidiuretic hormone from the hypothalamus/hypophysis, and suffer from hereditary diabetes insipidus (Valtin & Schroeder, 1964; Harrington & Valtin, 1968). It was anticipated that if the antidiuretic hormone is the major determinant of opiate-induced antidiuresis, the Brattleboro rats should be insensitive to the acute effect of morphine and related opiates on urine production and composition. In addition to exploring the acute effects of morphine on the Brattleboro rats, it was of interest to examine the effects of chronic administration of morphine. It was reasoned that if opiates release antidiuretic hormone, no tolerance should develop to the urinary effects of morphine in the Brattleboro rat and no urinary effects should be seen upon abrupt opiate withdrawal produced by a challenge dose of naloxone, the opiate antagonist. The studies of chronic morphine administration were initiated bearing in mind the possibility that vasopressin might be of importance in the consolidation of morphine tolerance (Krivoy, Zimmerman & Lande, 1974; de Wied & Gispen, 1976; Van

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Wimersma Greidanus, Kon Fat-Bronstein & Van Ree, 1978).

Methods

Animals and drugs used

Female homozygous Brattleboro rats (150 to 180 g) were purchased from Blue Spruce Farms (Altamont, N.Y.). Sprague-Dawley rats (250 to 280 g) were obtained from Simonsen (Gilroy, CA). Animals were kept at 24 to 25°C in a 12h light-dark cycle (06 h 00 min to 18 h 00 min). Four animals were kept per cage, food and water were allowed *ad libitum*. Due to the copious urine production of the Brattleboro rats, the bedding in the plastic cages was changed every 12 h.

Morphine sulphate was purchased from Malinckrodt Chemical Co. (St. Louis, MO). β-Endorphin was a generous gift from Professor C. H. Li, San Francisco, CA.

Animal hydration, urine collection and electrolyte analysis

Urine outflow was studied according to the procedure outlined by Huidobro & Huidobro-Toro (1979). In summary, rats were hydrated orally with 25 ml/kg tap water followed an hour later by 50 ml/kg 0.5% w/v NaCl solution. Immediately after the first oral hydration, rats were placed in metabolic cages provided with food pellets and 400 ml tap water. Urine was collected in 20 ml graduated cylinders; the volume of urine obtained following drug or saline administration was recorded cumulatively. The concentration of Na^+ and K^+ in the urine specimens was determined by flame photometry using conventional methods as described by Huidobro-Toro *et al.* (1979). Urine volume was expressed as ml of urine eliminated during the first hour following the opiates. The electrolytes are expressed as micro equivalents of Na^+ and K^+ excreted per hour (μEq/h).

Drug administration

Morphine and β-endorphin were injected into the right lateral cerebral ventricle (i.c.v.) of Brattleboro or Sprague-Dawley rats. Animals were briefly anaesthetized with ethyl ether for incision of the skull on the coronal suture, 2 mm to the right of the bregma to facilitate needle penetration. Injections were made with a 27 gauge needle; the volume of injection was 10 μl/rat. Previous control experiments demonstrated a uniform distribution of a dye into the ventricular space. The i.c.v. injections were made 10 min before the second liquid load. Doses are expressed as μg morphine or β-endorphin per rat. Control groups were injected with 10 μl sterile saline i.c.v. The solvent for all drugs administered i.c.v. was sterile saline. The selection of doses of opiates was based on previous experiments (Huidobro-Toro, *et al.*, 1979; Huidobro & Huidobro-Toro, 1979).

In separate groups of Brattleboro rats, morphine was injected (i.p.) in doses of 5.7 and 19 mg/kg. As a control, saline was injected (i.p.) in a volume of 1 ml/kg. Each group was composed of 6 rats each.

To study the effect of morphine after chronic administration of the opiate, five rats were rendered tolerant-dependent on morphine by the implantation of 75 mg morphine tablets on the subcutaneous tissue of the back, according to Way, Loh & Shen (1969). Rats were implanted on day zero with one morphine tablet, two on day one, and three on day two. Seventy-two hours after the first implant, rats were challenged with 19 mg/kg morphine (i.p.). As controls, a paired group of five rats was implanted with placebo pellets and challenged with 19 mg/kg morphine (i.p.). To precipitate morphine withdrawal, rats were injected with 1 mg/kg naloxone (s.c.) 96 h after the first implant; behavioural signs and urine outflow were recorded for 30 min after naloxone. The morphine or placebo pellets were not removed at the time of the challenge dose of morphine nor when withdrawal was precipitated by naloxone.

Statistical analysis

Results were analyzed by Dunnett's Tables (Dunnet, 1964) for multiple comparisons with a control, or Student's *t* test to compare results between morphine- and placebo-treated rats. Statistical significance was set at P less than 0.05.

Results

Urine production in the Brattleboro and Sprague-Dawley rats

Brattleboro rats exhibited a profuse diuresis; the volume of urine eliminated in 24 h by a group of 8 rats ranged between 50 and 150 ml, and the water consumption between 60 and 190 ml. Figure 1 compares the water intake and urine outflow in a group of 8 Brattleboro and Sprague-Dawley rats used in this study. The Brattleboro rats urinated about 36 times more than the Sprague-Dawley rats on a weight basis. The water intake in the Brattleboro rats was proportionally increased as compared to that of the Sprague-Dawley rats.

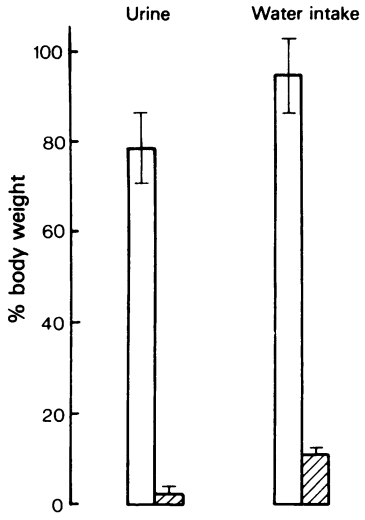

Figure 1 Water intake and urine production in Brattleboro and Sprague-Dawley rats. Groups of 8 rats were placed in metabolic cages and the 24 h water intake and urine production was determined. Results are expressed as a percentage of the body weight. (The mean body weight of Brattleboro rats was 145.8 ± 4.2 g and of the Sprague-Dawley rats, 299.1 ± 12.1 g). Columns indicate the mean percentage, bars the s.e. mean, open columns represent Brattleboro rats, hatched columns refer to Sprague-Dawley rats.

Effect of β-endorphin and morphine on urine formation in Brattleboro and Sprague-Dawley rats

The i.c.v. administration of 2 μg (0.58 nmol) β-endorphin to Brattleboro rats caused a significant and greater than 50% reduction in urine outflow and in the excretion of urine electrolytes as compared to saline-treated controls (Table 1). The i.c.v. injection of 1.9 μg (6.67 nmol) morphine/rat also caused oliguria and a reduction in the rate of Na^+ excretion, but no effect was observed on the rate of K^+ excretion. As an internal control for this set of experiments, Sprague-Dawley rats were injected i.c.v. with the opiates (the weight of these rats was about double that of the Brattleboro). β-Endorphin and morphine produced a 62 and a 42% reduction of the urine outflow respectively and about an 80 to 90% decrease in the excretion of Na^+ and K^+ (Table 1). The urine of the Brattleboro rats was markedly hypotonic as compared to that of the Sprague-Dawley rats. The concentrations of Na^+ and K^+ were found to be approximately 75 to 80% lower in the Brattleboro rats. These doses of the opiates produced some degree of narcosis and reduced the locomotion, but no catalepsy was evident. The behavioural effects lasted about 90 min.

Since the effects of the i.c.v. injection of morphine on urine composition were not clear, the narcotic was injected i.p. to a different group of Brattleboro rats. Morphine injected i.p. produced a dose-related decrease in urine outflow paralleled by a proportional reduction in the excretion rate of Na^+ and K^+ as compared to a saline-treated group of rats. With the dose of 19 mg/kg morphine, there was an almost complete inhibition of urine production 60 min after drug injection. The oliguria was coupled to a dramatic 90% reduction in the rate of excretion of Na^+ and K^+ (Figure 2). This effect of morphine lasted 4 to 5 h, after which the diuresis was not significantly different from that of the control, saline-treated group of Brattleboro rats.

Tolerance to the effects of morphine on urine formation in the Brattleboro rats

Tolerance developed to the effects of morphine on urine after chronic administration. Rats administered

Table 1 Effect of β-endorphin and morphine injected into the lateral ventricles of rats on urine outflow and composition

	n	*Volume* (ml/h)	*Na^+* (μEq/h)	*K^+* (μEq/h)
Brattleboro rats				
Saline	4	3.4 ± 0.8	108.15 ± 37.7	35.92 ± 12.9
β-Endorphin 2.0 μg	8	1.2 ± 0.5*	56.60 ± 15.8	16.89 ± 5.0*
Morphine 1.9 μg	5	1.3 ± 0.8	44.18 ± 19.0	34.35 ± 12.6
Sprague-Dawley rats				
Saline	5	4.0 ± 0.7	379.40 ± 34.2	205.1 ± 20.5
β-Endorphin 1.0 μg	7	1.5 ± 0.7*	59.47 ± 19.0**	67.51 ± 19.4**
Morphine 1.9 μg	5	2.3 ± 0.5	14.38 ± 4.1**	33.54 ± 11.01**

Rats were injected into the left lateral ventricle under light ether anaesthesia. The volume of each injection was 10 μl/rat. All rats were water loaded with 25 ml/kg tap water followed by 50 ml/kg 0.5% NaCl.
* *P* value less than 0.05; ** *P* value less than 0.01.

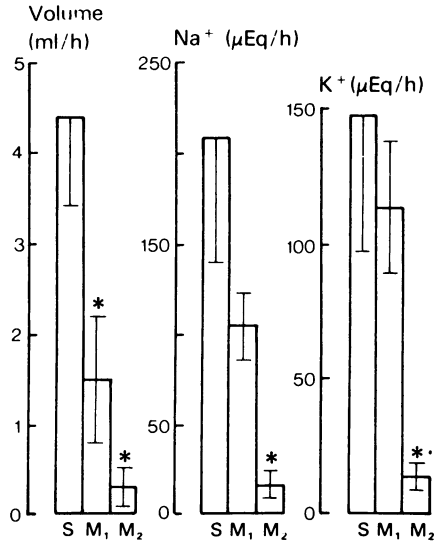

Figure 2 Effect of morphine on the urine outflow and excretion of urine electrolytes in Brattleboro rats. Three groups of six rats each were injected intraperitoneally with 5.7 (M1) or 19 (M2) mg/kg morphine or 1 ml/kg saline (S). Urine volume was measured 1 h after morphine; urine electrolytes were measured in a sample obtained in the 60 to 80 min period following the morphine injection. Urine volume is expressed as ml/h; electrolytes as μEq of Na^+ or K^+ per h (μEq/h). Columns refer to the mean values, bars to the s.e. mean, asterisks indicate to a P value less than 0.05.

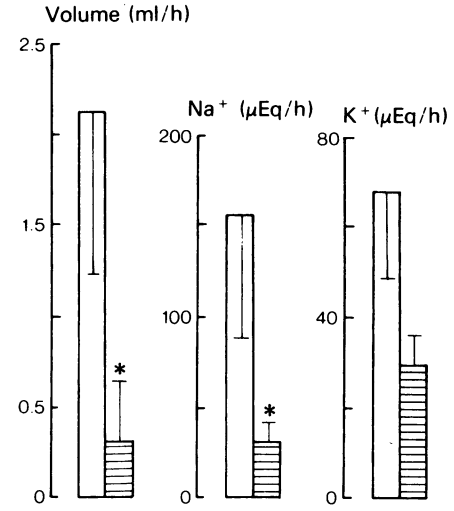

Figure 3 Effect of a challenge dose of morphine in Brattleboro rats chronically treated with morphine and placebo controls. Rats were chronically administered morphine by the technique of morphine pellet implantation. A control group was implanted with placebo pellets. Seventy-two hours following the first implant, both groups of rats were challenged with 19 mg/kg morphine (i.p.) after water loading. The urine outflow was measured during the first hour following the narcotic and expressed as ml/h. Urine electrolytes were determined in a sample obtained in the 60 to 80 min interval following morphine; results are expressed as μequivalents per hour (μEq/h). Open columns refer to the mean value obtained in the morphine-treated group, hatched columns to the placebo-treated group. Bars indicate s.e. mean. Asterisks indicate a P value less than 0.05.

morphine chronically for 72 h were markedly less sensitive to the antidiuretic effect as well as to the reduction of urine electrolyte excretion produced by a challenge dose of 19 mg/kg morphine. The placebo-treated group injected with 19 mg/kg morphine manifested an intense antidiuresis paralleled by a reduced excretion of urine electrolytes (Figure 3). Curiously, 24 h after the challenge dose, the morphine-treated group produced 114.4 ± 21.9 ml urine ($n = 5$) as compared to 170.6 ± 14.4 ml ($n = 5$) for the placebo-treated group ($P < 0.05$).

Development of physical dependence to morphine in Brattleboro rats

A challenge dose of 1 mg/kg naloxone (s.c.) precipitated an intense opiate withdrawal syndrome in rats implanted with morphine for 3 days. The abrupt withdrawal was characterized by vigorous jumping, teeth chattering, hypermotility, intense defaecation and diuresis. The tolerant-dependent group of rats urinated 6.0 ± 2.8 ml during the 30 min period post-naloxone as compared to 1.6 ± 0.7 ml in the placebo-implanted group. No behavioural effects were noted in the placebo group after treatment with naloxone.

Discussion

The Brattleboro rats used in this investigation suffered from a severe condition of diabetes insipidus. Apart from the profuse diuresis and the corresponding large water intake, the urine was hypotonic compared to that of Sprague-Dawley rats. These data indicate their lack of antidiuretic hormone (Valtin & Schroeder, 1964; Harrington & Valtin, 1965; 1968). Even though no direct evidence is provided as to the extent of the deficiency in antidiuretic hormone in these particular rats, the hypotonic urine and the diuresis state the severity of the pathological condition.

Morphine and β-endorphin caused a decrease in the urine output and in the rate of excretion of Na^+

and K^+ in the Brattleboro as well as the Sprague-Dawley rats. The effect of the endogenous opioid peptide and morphine on urine production in the Brattleboro rats is qualitatively comparable to results obtained by other investigators using Sprague-Dawley rats (Tseng, Wei, Loh & Li, 1978; Bisset, Chowdrey & Feldberg, 1978; Huidobro-Toro, *et al.*, 1979). However, it is apparent that the Brattleboro rats require a dose of the opiates about 2 to 3 fold higher than the Sprague-Dawley rats to cause a similar reduction in the excretion of Na^+ and K^+. This quantitative difference is probably related in part to the fact that these rats have a hypotonic urine that could mask the total effect of the opiates on urine composition. It is of importance to emphasize, that at the doses of the opiates used, morphine or β-endorphin injected centrally or peripherally caused a comparable antidiuresis in both the Brattleboro and Sprague-Dawley rats (Huidobro, 1978; Huidobro-Toro *et al.*, 1979). This fact argues that if the Brattleboro rats are slightly less sensitive to the total effect of opiates on urine composition, they are still markedly responsive to antidiuresis, in contrast to the almost complete lack of antidiuretic hormone reported for the Brattleboro rats.

It was of interest to discover whether tolerance developed to the antidiuretic effect of morphine after chronic opiate treatment in Brattleboro rats. A previous study by De Wied & Gispen (1976) demonstrated that homozygous Brattleboro rats had a delayed development of tolerance to morphine analgesia unless the rats were supplemented with injections of arginine vasopressin. However, in our experiments no evidence was found to substantiate this hypothesis since tolerance developed to the effects of morphine on urine formation. In addition, rats chronically treated with morphine were found to be dependent on morphine as evidenced by an intense withdrawal syndrome upon challenge with naloxone in agreement with previous reports in mice or rats (Huidobro & Maggiolo, 1961; Way *et al.*, 1969; Wei, Loh & Way, 1973; Huidobro, 1978). Our results support the recent investigations of Schmidt, Holaday, Loh & Way (1978) who found that vasopressin or oxytocin failed to antagonize the acute effects of morphine in mice and did not facilitate narcotic tolerance development. It is possible that some of these discrepancies are due to the different schedules of morphine administration, since after pellet implantation, there is a sustained release of morphine during the time of the implant (Maggiolo & Huidobro, 1961).

The mechanism of action of the antidiuresis caused by morphine is not clear. It has been repeatedly argued that morphine releases antidiuretic hormone from the hypophysis explaining the water retention (De Bodo, 1944). However, this link has yet to be clearly demonstrated. The present results indicate that morphine and β-endorphin cause a drop in the urine outflow and a reduction in the excretion of urine electrolytes in the Brattleboro rats depiste the severe condition of diabetes insipidus. These results suggest that the antidiuretic hormone is not the sole determinant of the morphine antidiuresis in rodents. A similar conclusion was arrived at by Fujimoto who mentions that the morphine antidiuresis persisted in Brattleboro rats, though no data were presented in his review (Fujimoto, 1971). George & Way (1959) found that lesions of the hypothalamus which produced diabetes insipidus blocked the antidiuretic response of 0.1 mg/kg morphine (i.p.), but failed to inhibit the response to doses of 1 to 10 mg/kg morphine. If the release of antidiuretic hormone were the cause of the morphine effect, rats with an almost total lack of vasopressin would be expected to be completely unresponsive to morphine. This experimental evidence supports our view that apart from the antidiuretic hormone, other variables should be considered to explain fully the effect of opiates on urine formation. It is possible that morphine through a central and/or a peripheral mechanism diminishes glomerular filtration, determining a drop in kidney perfusion and urine production. In support of this contention, it is well established that morphine and related narcotics as well as the opioid-like peptides injected systematically or into the cerebral ventricles produces a fall in peripheral blood pressure (Schideman & Johnson, 1948; Handley & Keller, 1950; Winter, Gaffney & Flataker, 1954; Baker & Woods, 1957; Mills & Wang, 1964; Huidobro & Huidobro, 1970; Laubie, Schmitt, Vincent & Remond, 1977; Feldberg & Wei, 1977; Bolme, Fuxe, Agnati, Bradley & Smythies, 1978). Recently, Huidobro *et al.* (1979) showed that acute morphine reduced the clearance of endogenous creatinine, suggesting that the acute administration of morphine decreased the glomerular filtration rate. Thus, an opiate-mediated cardiovascular alteration could be partially responsible for the narcotic-induced antidiuresis.

In summary, present results do not completely exclude the participation of antidiuretic hormone in the rat's opiate-induced antidiuresis, but strongly suggest that other variables are involved. It is suggested that a cardiovascular effect could operate concomitantly and be of primary importance in determining the antidiuretic effect of the opiate drugs.

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