Br. J. Pharmac. (1978), **64**, 167–171.

ANTIDIURETIC EFFECT OF MORPHINE IN THE RAT: TOLERANCE AND PHYSICAL DEPENDENCE

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1 Injection of rats with morphine or methadone, before they received a water load equivalent to 5% of their body weight, produced a dose-dependent antidiuretic effect. Following the antidiuresis, urine was eliminated with kinetics similar to control untreated rats.

2 The antidiuretic effect of morphine or methadone was blocked by naloxone administered before the opiate, or reversed when given after the opiate.

3 Rats implanted with morphine pellets developed a marked degree of tolerance to the antidiuretic effect of morphine. Tolerance was also obtained on injection of three daily doses of morphine or methadone over two days.

4 Withdrawal symptoms were precipitated by naloxone in rats implanted with pellets of morphine; under these conditions the animals showed a marked reduction in urine production as compared to naive rats.

Introduction

Morphine and its surrogates modify urine production. Some investigators describe an antidiuretic action (De Bodo, 1944; Papper and Papper, 1964; Fujimoto, 1971) and others a diuretic effect of morphine (Boyd & Scherf, 1940; Singh, Khana, Srivatava, Lae, Roy & Subramanyan, 1969; Marchand, 1970). The antidiuretic action has been attributed to the release of antidiuretic hormone by morphine (De Bodo, 1944; George & Way, 1959; Fujimoto, 1971); however, this opinion is not shared by all the investigators (Boyd & Scherf, 1940; Handley & Keller, 1950; Schnieden & Blackmore, 1955).

These discrepancies may be attributed to the fact that the liberation of antidiuretic hormone may not be the only mechanism involved in morphine antidiuresis. Renal haemodynamic changes (Papper & Papper, 1964), nervous influences (Lipschitz & Stokey, 1947), species differences (Inturrisi, May & Fujimoto, 1968), inhibition of water absorption from the gastrointestinal tract (Schnieden & Blackmore, 1955), urine retention in the bladder (Inturrisi & Fujimoto, 1968), changes in electrolytes and the use of hydrated and non-hydrated animals (for reference, see Fujimoto, 1971) may produce considerable changes in water distribution. However, if rats are previously loaded with water, morphine will produce a consistent antidiuretic response (Fujimoto, 1971). The aim of this paper was not to investigate the validity of the diverse hypotheses put forward to explain the production of morphine antidiuresis, but to discover whether rats could serve as an appropriate model for the investigation of the action of opiates in urine production.

Methods

Sprague-Dawley rats of the strain developed at the Institute, weighing 180 to 210 g, were used. Animals were housed 5 rats to a cage and maintained at 22 to 23°C. The vivarium was artificially illuminated during 14 h of the day. Rats were fasted 18 h before the experiments, but allowed free access to tap water.

Experiments began normally at approximately 09 h 00 min. Each rat was placed in an individual metabolic cage (15 × 25 × 13.5 cm) for urine collection. The procedure outlined by George & Way (1959) was followed. Briefly, animals were hydrated twice: first, rats received by gastric tube a volume of tap water equivalent to 2.5% of the body weight; 60 min later, rats were given a second load, this time of distilled water, equivalent to 5% of body weight. Following the second hydration, urine was collected and measured cumulatively every 20 min during a 120 to 180 min period. Narcotics or other drugs were always injected intraperitoneally before the second water load (2nd WL). When naloxone was used, it was generally injected intraperitoneally before the narcotics or the 2nd WL. Naive animals were injected with 0.9% w/v NaCl solution (saline) instead of drugs;

these rats are referred to as controls. One control group was used for each experimental series. Each rat was used in only one experiment.

Drugs used were: morphine hydrochloride, methadone hydrochloride and sodium hydrochloride from Merck Chemical Co.; and naloxone supplied through the generosity of Endo Laboratories. The dose of the drug (given in terms of the base) was dissolved in distilled water and injected intraperitoneally in a volume of 1 ml/kg.

Tolerance and induction of tolerance

In this paper, tolerance is defined as a statistically significant decrease in the antidiuresis produced by a challenge dose of opiate, as compared to the response of the opiate in a control rat pretreated with saline, independent of the number of morphine administrations or schemes of treatment. Tolerance was established mathematically by a shift to the right in the dose-effect curve. The following schemes of treatment were used to produce tolerance and physical dependence: (a) In these experiments only one injection of morphine was used: a priming dose of 10, 20, 30 or 40 mg/kg of morphine was injected 0.5, 1, 2, 4, 6, 12, 18, 24 or 42 h before the 2nd WL. An increase of urine elimination indicated the development of tolerance; (b) On day 0, several groups of naive rats were injected with a priming dose of 10, 20, 30 or 40 mg/kg of morphine. Animals were challenged with the same dose of the narcotic 1, 2 and 3 days after the priming injection of the alkaloid and before the 2nd WL. Two doses of morphine were administered in these experiments, one to induce and the other to test if the first dose had produced tolerance; (c) Three daily injections of morphine were administered: rats were injected with a priming dose of 10, 15 and 15 mg/kg of morphine at 08 h 00 min, 12 h 00 min and 16 h 00 min; on the second day the treatment was the same but the doses of morphine were: 20, 20 and 30 mg/kg. On the third day rats were challenged with 10 mg/kg of morphine before the 2nd WL. Methadone was employed according to schemes of treatment very similar to those used for morphine: the priming dose varied from 2.5 to 7.5 mg/kg and the drug was injected 15 min to 20 h before the 2nd WL; (d) Pellet implantation. Rats were implanted subcutaneously with three morphine pellets on day 0. Each pellet contained 100 mg of morphine base highly compressed. On the 4th day after the implant, four additional pellets were administered to each rat. At various intervals (1 to 7 days), rats were challenged with 10 to 80 mg/kg of morphine to test if tolerance to the antidiuretic effect had developed.

Results were calculated as cumulative percentages of the volume of the 2nd WL eliminated as urine and expressed as the mean (mean ± s.e.) of each experimental group (5 males and 5 females). For statistical analysis Student's t test was used. Significance was set at a level of $P < 0.05$; in general, a difference of at least 12% was necessary to obtain this level of significance.

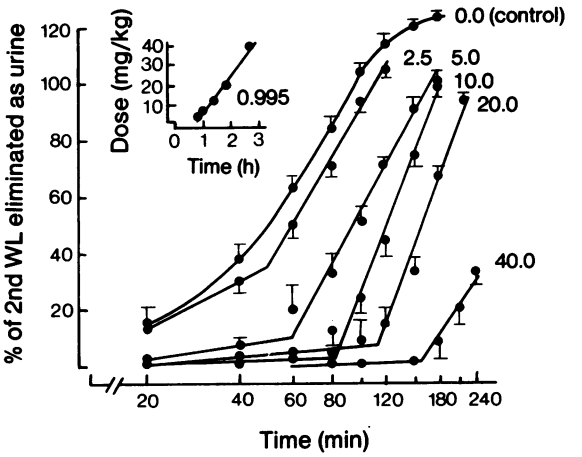

Figure 1 Time course of antidiuretic effect of morphine. The antidiuretic effect of morphine was followed by a rapid phase of urine elimination. The duration and intensity of the antidiuresis depended on the dosage of morphine as illustrated in the inset. The correlation coefficient obtained was 0.995. In this and in the other figures each point represents the mean values obtained from 5 male and 5 female rats. The vertical bars denote the s.e.; the numbers indicate the dose of morphine in mg/kg employed in each experiment. Abscissa scale is logarithmic. (Standard errors have been omitted in the other figures to avoid confusion. In general, differences of at least 12% attained a level of $P < 0.05$.)

Results

Effects of morphine and methadone on the elimination of the second water load

When saline was injected 5 to 10 min before the 2nd WL, the control curve shown in Figure 1 was obtained. A volume of urine equivalent to the 2nd WL was eliminated in about 120 min. When morphine or methadone were administered instead of saline, a period of almost complete antidiuresis was evident after which the 2nd WL was rapidly eliminated. The time course of the morphine antidiuresis was proportional to the dose of morphine (Figure 1) and showed a good correlation coefficient between both variables ($r = 0.9955$). After 40 mg/kg of morphine only 30% of the 2nd WL was eliminated during

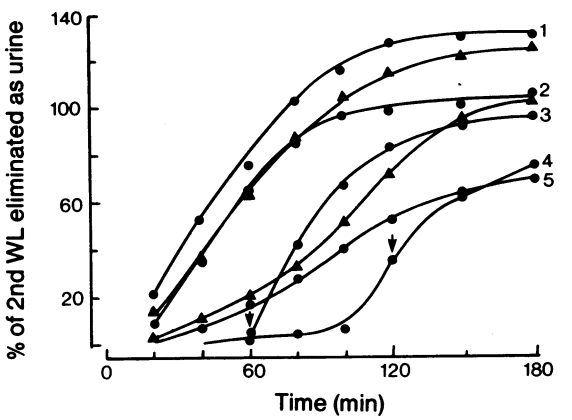

Figure 2 Effect of naloxone on morphine antidiuresis. Upper line denoted by (▲): urine elimination in naive rats after a 2nd WL. Lower line denoted by (▲): urine elimination after the injection of 5 mg/kg of morphine before the 2nd WL. (1) Naloxone (10 mg/kg) 10 min before morphine (10 mg/kg) and immediately before the 2nd WL. (2) Effect of naloxone (10 mg/kg) on urine elimination injected before the 2nd WL. (3) Effect of naloxone (10 mg/kg) injected at the arrow, 60 min after morphine, on urine elimination. Note the almost immediate antagonism. (4) The same as (3) but naloxone was injected (at the arrow) 120 min after morphine (10 mg/kg). (5) Urine eliminated during an abrupt withdrawal syndrome precipitated by naloxone (10 mg/kg) in rats implanted with pellets of morphine.

4 h. Doses of morphine higher than 40 mg/kg could not be studied because of toxic effects; 35% of the rats died at this dose level.

Methadone in doses of 2.5 to 7.5 mg/kg produced the same effect as morphine.

Effect of morphine on the elimination of sodium chloride load

When the 2nd WL was substituted by solutions of sodium chloride of different concentrations (0.5; 0.75; 1.0 or 2.0%), the volume of urine eliminated during a period of 210 min following the 2nd WL was always less than 100% of the load and volume was not proportional to the concentration of the salt solution administered. When morphine (2.5, 5.0, 10.0 or 20.0 mg/kg) was injected 5 to 10 min before the second load, equivocal results were obtained.

Effect of naloxone on the antidiuresis produced by morphine

Naloxone (10 mg/kg) did not modify the elimination of the 2nd WL (Figure 2), implying that it does not have agonistic properties. When naloxone (10 mg/kg) was administered 5 min before morphine (5 mg/kg) and the 2nd WL, the curve illustrated in Figure 2 was obtained: naloxone counteracted completely both intensity and duration the effect of 5 mg/kg of morphine. The urine elimination was practically the same as that observed in the control condition.

Since naloxone blocked the antidiuretic effect of morphine, it was of interest to study if the drug could also reverse the antidiuresis produced by the alkaloid. Two sets of experiments were performed. In the first set, 10 mg/kg of naloxone was administered 60 min after a dose of 10 mg/kg of morphine; that is, when the antidiuretic effect of the opiate was intense. As shown in Figure 2, naloxone rapidly reversed the effect of morphine, showing an almost complete reversal of the antidiuresis. In the second set of experiments, naloxone was administered 120 min after 10 mg/kg of morphine; i.e., when the antidiuretic effect of morphine was over and diuresis had begun. Under these conditions, naloxone did not accelerate the rate of diuresis (Figure 2).

Rats implanted twice with 3 and 4 pellets of morphine (days 0 and 4 of a week), received naloxone (10 mg/kg i.p.) immediately before a 2nd WL on day 7 after the first implant. The rats exhibited a peculiar withdrawal syndrome after the drug, characterized by hypermotility, a profuse salivary secretion and intense stereotyped movements of short duration that resembled the activity of these animals when digging. Apart from the well known vegetative signs, there was an exaggerated retention of urine after the 2nd WL (Figure 2).

Naloxone (10 mg/kg) also blocked the antidiuretic effect produced by different doses of methadone (2.5 to 7.5 mg/kg).

Development of tolerance to the antidiuretic effect of morphine

Injections of morphine. Rats were injected with one or two doses of morphine according to the technique described in Methods. If water elimination was greater than that produced after 10 mg/kg of morphine, it was accepted that tolerance had developed (see Methods). The results were not uniform, some experiments were negative and others positive (Figure 3). Negative results were mainly observed when two morphine injections were used, one to produce tolerance and the other to reveal if tolerance had developed. Neither the dose of morphine nor the time elapsing between the administration of the opiate and the 2nd WL seemed to be important in the development of tolerance.

Three daily injections schedule. After three daily injections of morphine over two days (see Methods)

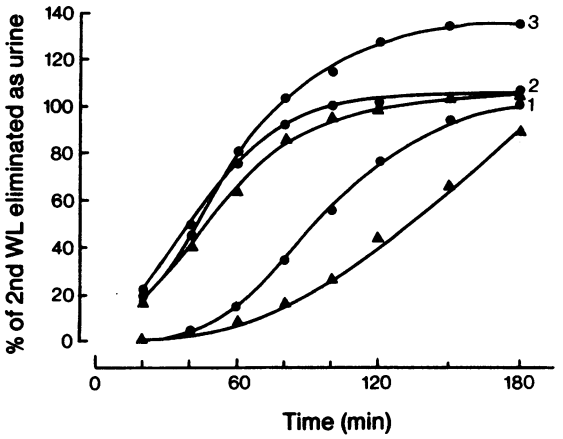

Figure 3 Tolerance to morphine antidiuresis induced by a single dose of morphine. Upper line denoted by (▲): urine elimination after the 2nd WL. Lower line denoted by (▲): urine elimination after 10 mg/kg of morphine injected immediately before the 2nd WL. Effect of 10 mg/kg of morphine injected 0.5 (curve 1), 12 (curve 2) and 24 h (curve 3) before the 2nd WL.

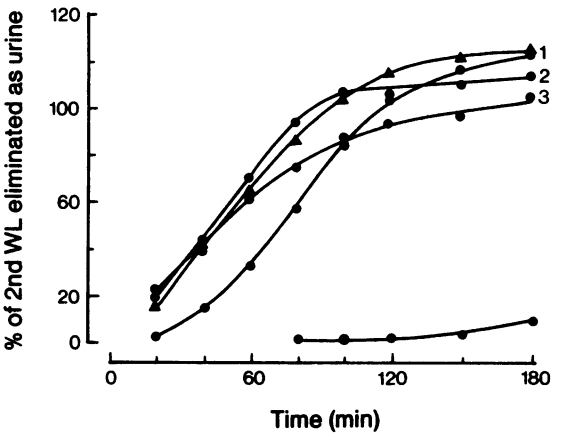

Figure 4 Tolerance to the antidiuretic effect of morphine induced by morphine pellet implantation. Line denoted by (▲): urine elimination after the 2nd WL. (1) Effect of 80 mg/kg of morphine on rats implanted with 7 morphine pellets for one week. (2) Effect of 10 mg/kg of morphine in rats implanted with 3 pellets of morphine 4 days previously. (3) Effect of 10 mg/kg of morphine in rats implanted with 3 morphine pellets 1 day before. (4) The effect of 40 mg/kg of morphine before the 2nd WL in naive control rats.

morphine induced tolerance but it was quantitatively inferior to that observed after morphine pellet implantation.

Pellet implantation. Tolerance to the antidiuretic effect of morphine was evident as early as 24 h after the implantation of three pellets of morphine. Tolerance was marked four days after the implant. These results are illustrated in Figure 4. A more marked degree of tolerance could be demonstrated a week after seven pellets. As can be observed in Figure 4 a dose of 80 mg/kg of morphine (which proved to be 100% lethal in naive control rats) produced no death and had no antidiuretic effect. Compare these results with the antidiuretic effect of 40 mg/kg of morphine in naive rats (Figures 1 and 4).

Discussion

Administration of morphine after the 2nd water load produced an intense antidiuresis, followed by a secondary diuretic phase which corresponded to the aqueous elimination of the antidiuretic phase. The larger the dose of morphine the longer was the duration of the antidiuresis. The transition of one phase to the other was rapid, occurring from 60 to 120 min after the injection of 5 to 20 mg/kg of the narcotic. The shortness of this period may be due to the fact that by the first hour after a single dose of morphine or methadone, the cerebral concentrations of these drugs had decreased considerably (Misra, Mitchell & Woods, 1971; Misra & Mulee, 1972).

The slope of the eliminatory phase was similar to that observed in the control curve of the naive rats (Figure 1), possibly indicating a similar mechanism of diuresis in both cases. Larger doses of morphine produced a prolonged eliminatory phase, perhaps due to a new redistribution of water as a consequence of a prolonged antidiuretic period. Experiments are in progress to evaluate the involvement of the antidiuretic hormone in this opiate effect.

Naloxone had no antidiuretic action. When the drug was injected during the antidiuretic period of morphine or methadone, it reversed the antidiuresis to an almost immediate diuresis. If naloxone was injected during the water elimination phase, the drug had no effect, probably because the narcotic had already been redistributed or metabolized.

Rats rapidly develop tolerance to the antidiuretic action of morphine (Inturrisi & Fujimoto, 1968; Fujimoto, 1971). The implantation of pellets or the administration of three daily doses of morphine produced tolerance to the antidiuretic effect, a result in accordance with those of Inturrisi & Fujimoto (1968). When one or two injections of morphine were

employed, tolerance did not develop, unlike the results obtained in mice when the hot plate technique was used (Huidobro, Huidobro-Toro & Way, 1976). The time course and the dose required to induce single dose tolerance may be different in each case.

The present results show that narcotic analgesics interact with the opiate receptor to produce antidiuresis in the rat. It is concluded that water diuresis in the rat is a suitable model for studying the actions of opioids on urine production.

This research was partially supported by grant 215/75 of the Catholic University of Chile Research Fund. The author wishes to express gratitude to J.P. Huidobro-Toro for his assistance and valuable suggestions in the preparation of the manuscript. He is also indebted to R. Miranda for technical assistance.

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(Received September 6, 1977. Revised May 18, 1978.)