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Impaired Development of Tolerance to Morphine Analgesia in Rats with Hereditary Diabetes Insipidus

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Abstract. Recently it was reported that vasopressin facilitates the development of resistance to the analgesic action of morphine. Therefore, the development of tolerance to daily administration of morphine-HCl (10 mg/kg i.p.) was studied in a series of trials on a hot plate using rats with hereditary diabetes insipidus (DI), which lack the ability to synthesize vasopressin. In contrast to heterozygous DI rats, who developed full tolerance after the fifth injection, homozygous DI rats showed a delayed development of tolerance. Substitution of HO-DI rats with either arginine-8-vasopressin (3 μg/rat, s.c. daily) or the endocrinologically inert fragment of vasopressin desglycinamide lysine-8-vasopressin (5 μg/100 g, s.c. daily) restored the impaired development of tolerance towards normal. The data support the notion that vasopressin is important to the development of tolerance to narcotic analgesics and that its mechanism of action is dissociated from its endocrine effect but rather resembles that of its known influence on memory consolidation.

Key words: Diabetes insipidus – Morphine tolerance – Vasopressin.

Introduction

Vasopressin and vasopressin analogues facilitate active avoidance behavior in hypophysectomized (Bohus *et al.*, 1972) and intact rats (King and de Wied, 1974) and increase resistance to extinction of active and passive avoidance behavior (de Wied, 1971; Ader and de Wied, 1972). These observations were interpreted indicating that vasopressin and vasopressin analogues are involved in learning and memory processes.

Recently, Krivoy *et al.* (1974) reported that a naturally occurring vasopressin analogue, desglycinamide lysine-8-vasopressin (DG-LVP) (Lande *et al.*, 1971) which has essentially similar behavioral effects as vasopressin but which is endocrinologically rather inert (de Wied *et al.*, 1972), facilitates the development of resistance to the analgesic action of morphine. This suggests that vasopressin is physiologically involved in the development of tolerance to narcotic analgesics. If this were true, rats with hereditary diabetes insipidus (DI; Valtin and Schroeder, 1964) which lack the ability to synthetize vasopressin might have difficulty in developing tolerance to the analgesic action of narcotic analgesics.

Methods

Brattleboro rats were obtained from the Central Breeding Laboratories TNO Zeist, The Netherlands, and both heterozygous (HE-DI) and homozygous (HO-DI) animals were tested. Male rats, weighing approximately 180–220 g were used. Morphine-HCl (M-Cl) was dissolved in 0.9% NaCl and administered intraperitoneally in a dose of 10 mg/kg (1 mg/ml). Synthetic desglycinamide lysine-8-vasopressin (DG-LVP) or arginine-8-vasopressin (AVP) was dissolved in a drop of 0.001 M HCl, further diluted with 0.9% NaCl. The behavioral procedure consisted of a series of trials on a hot plate according to Eddy and Leimbach (1953). The hollow copper plate placed in heat insulating material, was connected to a Colora Ultra thermostat NB/DS-8 4 waterbath, allowing the plate to be warmed to the temperature of choice thus ensuring a constant temperature throughout the experiment, within a range of less than ± 0.1°C. The rat was placed onto the heated plate within a restraining perspex cylinder (⌀ 19.5 cm) and its reaction to the heat stimulus through its feet was determined with a stopwatch. The criteria of acute discomfort for the rat were (a) licking of one paw (clearly distinguishable from spontaneous maintenance behavior), or (b) intensive jerking with lifting off or jumping on its hind legs. If not indicated otherwise, the experimentor terminated the trial if the response latency exceeded 30 s. Statistical analysis was performed using Mann Whitney *U*-test when comparing different treatment groups per day and a paired Wilcoxon rank-test when comparisons were made between days within one treatment group. Differences were assigned to be significant for values of $P \leq 0.05$ (two tailed).

Results

In pilot studies it was found that a reproducible analgesic effect of M-Cl (10 mg/kg, 30 min prior to the test) was obtained if predrug, initial reaction times were in the order of 8–10 s. Therefore, all rats were tested 1 day prior to the beginning of the drug experiment at various temperatures of the hot plate to

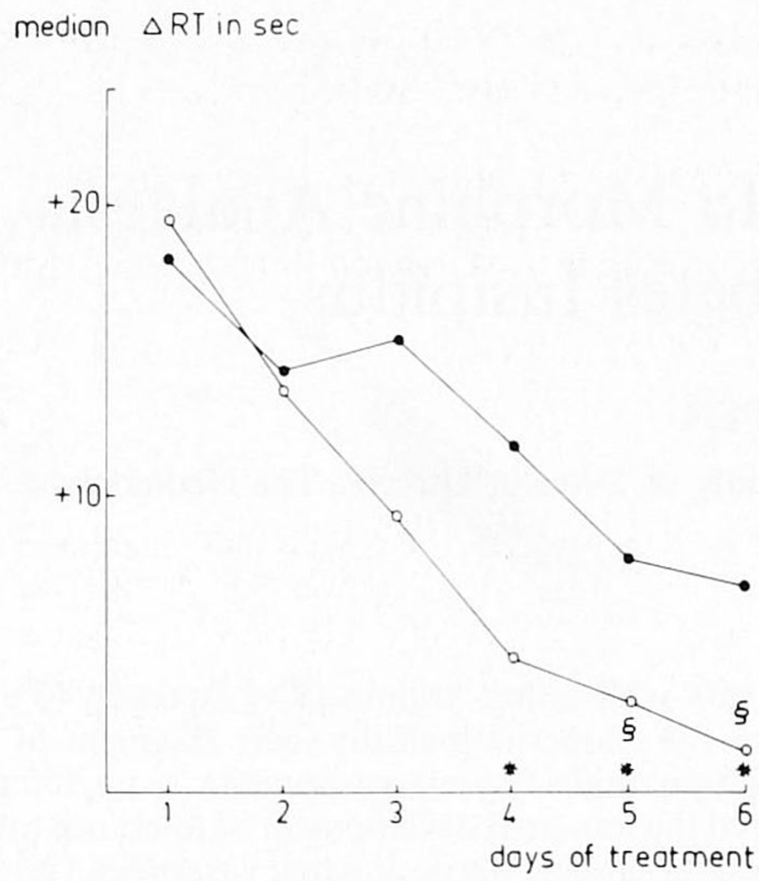

Fig. 1. Development of tolerance to morphine analgesia in Brattleboro rats. Heterozygous (○——○), $n = 10$) and homozygous (●——●, $n = 10$) diabetes insipidus rats. All rats were treated daily with morphine (10 mg/kg i.p.) and tested 30 min later on hot plate. Median values of ΔRT [*i.e.* reaction time (RT) minus initial reaction time (IRT)] are plotted vs. days of treatment. * $P < 0.05$ Ho vs. He. § No significant difference between RT and IRT within treatment group

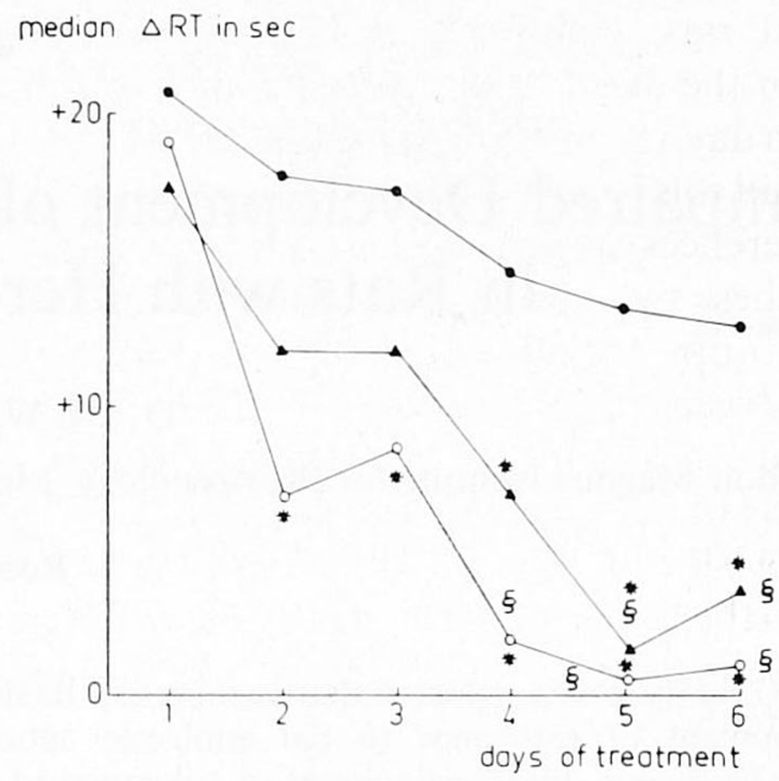

Fig. 2. Effect of vasopressin on development of tolerance to morphine analgesia in Brattleboro homozygous diabetes insipidus rats. All rats were treated daily with morphine (10 mg/kg i.p.) and tested 30 min later on hot plate. Immediately after testing on hot plate 1 group received daily saline (s.c., $n = 6$; ●——●); a second group DG-LVP (5 µg/100 g, s.c., $n = 7$, ▲——▲), and a third group AVP (3 µg/rat s.c., $n = 6$, ○——○) (ΔRT = RT − IRT). * $P < 0.05$ peptide treated group vs. saline. § No significant difference between RT and IRT within treatment group

determine their response latency. There is a small but significant difference in responding to heat stimulation between HE-DI and HO-DI rats. HO-DI rats appeared to be slightly more sensitive than HE-DI rats to heat stimulation. The HO-DI rats reacted after a significantly shorter interval (*ca.* 2 s) to temperature ranging from 50.5 to 52.0° C. Accordingly, HO-DI rats were tested at a temperature approximately 1.0° C lower than that used for HE-DI rats, resulting in similar initial reaction times. A single dose of 10 mg/kg M-Cl caused a marked delay in response on the hot plate in both HO-DI and HE-DI animals which was apparent at least throughout the first 90 min after the injection. The effect had disappeared 20 h after the M-Cl injection and therefore no difference between HO-DI and HE-DI rats was found: thus, the performance of the two groups of rats on the hot plate after acute morphine was essentially the same.

In subsequent experiments the effect of daily treatment with M-Cl on the response latency was studied in order to measure the development of tolerance to the M-Cl injection. On day I, the initial reaction time (IRT) was determined before and 30 min after a single i.p. dose of M-Cl (10 mg/kg). The following days, always at the same time of the day, the animals were injected with the same amount of M-Cl, and tested 30 min later on the hot plate. The response to daily treatment with M-Cl diminished in time in HE-DI rats as expected. Full tolerance to M-Cl had developed after the fifth injection (Fig. 1). The response in HO-DI rats differed markedly from that of the HE-DI group *i.e.* reduction in response latency developed at a lower speed in these animals. Morphine-induced latencies in reaction times were significantly larger in HO-DI rats at days IV, V, and VI as compared to those in HE-DI rats. The drug reaction times on each of the 6 days differed significantly from the IRT values obtained on day I.

A similar experiment was performed with 9 HO-DI rats and essentially the same results were obtained (median RT = 11 s; median RT after the first M-HCl injection = 21 s; median RT after 8 M-HCl injections = 18 s) again showing the delay in development of tolerance to M-Cl in HO-DI rats.

In a subsequent experiment HO-DI rats were treated with DG-LVP or AVP in combination with daily treatment with M-Cl. DG-LVP is a vasopressin analogue which is almost devoid of classical endocrine activities (de Wied *et al.*, 1972) and which was found to facilitate development of resistance to morphine analgesia (Krivoy *et al.*, 1974). The experimental design was similar to that used in the previous experiments (Fig. 2). HO-DI rats were randomly divided into three groups receiving either DG-LVP (5 µg/100 g), AVP (3 µg/rat), or saline s.c. immediately after each trial. In this experiment HO-DI rats responded with shorter latencies if treated with DG-LVP or AVP in contrast to saline treated controls (Fig. 2). Treatment

of HO-DI rats with AVP in addition to morphine resulted in the development of resistance to the analgesic from day IV on. Similar treatment with DG-LVP induced full tolerance as of day V. However, no significant differences were found in daily reaction times between these two groups. In both morphine/peptide-treated groups significantly shorter reaction times were observed in comparison to morphine/saline-treated rats, beginning at the second and fourth day respectively of treatment. Chronic treatment of HO-DI rats with AVP or DG-LVP did not affect their responsiveness to the hot plate since no differences were found between saline/saline and saline/DG-LVP or saline/AVP-treated rats.

Discussion

The data indicate that lack of vasopressin interferes with the development of tolerance to morphine analgesia (Fig. 1) which can be restored by either the administration of vasopressin (AVP) or its endocrinologically inert analogue DG-LVP (Fig. 2). It is highly unlikely therefore that the normalizing influence on the development of tolerance to M in HO-DI rats is caused by normalization of water homeostasis. Narcotic analgesics might be processed differently in the Brattleboro HO-DI and HE-DI rats. However, the fact that a single injection of morphine in both groups resulted in a similar analgesic response with respect to amplitude and duration favors the notion that the difference between the two types of rats relates more to their ability to develop tolerance to chronic treatment with morphine than to an alteration in metabolic degradation. Vasopressin and vasopressin analogues are also involved in memory processes. HO-DI rats have a serious deficit in memory consolidation which can be resorted by treatment with AVP or DG-LVP (de Wied *et al.*, 1974). Similar peptides increase resistance to extinction of conditioned avoidance behavior in intact rats. Interestingly, DG-LVP protects against puromycin-induced memory blockade in mice (Lande *et al.*, 1972) and tolerance to the analgesic action of morphine can be inhibited by drugs which inhibit protein synthesis (Cox and Osman, 1970). Development of tolerance might be regarded as a form of learning or memory (Cohen *et al.*, 1965). Thus, similar mechanisms as in learning and memory processes may be involved. The influence of vasopressin (and analogues) therefore, may be of more general nature, *i.e.* to promote the storage of exogenous and endogenous information in the cell.

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