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Micturition in naive and morphine-dependent rats

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1 Voiding responses were recorded in conscious water-loaded rats. Morphine sulphate (5 mg kg^{-1}) elevated the volume threshold for micturition (MV); the group mean MV of 16 rats after morphine was 40% larger than control. Micturition was nevertheless complete since no urine remained in the bladder afterwards.

2 The implantation of 2 or 4 morphine-base pellets (150 or 300 mg morphine) elevated for 12 days the MV in water-loaded rats. On the 3rd to the 10th day following implantation the group mean was approximately twice that of untreated controls. After micturition was over no residual urine was found in the bladder.

3 Within 3 days the rats became tolerant to the antinociceptive action of the morphine-base pellets but little apparent tolerance developed to their action on micturition.

4 On the 1st day after the pellets were removed, the mean MV was reduced. When withdrawal was precipitated by the administration of naloxone the MV was often too small to measure. This component of a withdrawal syndrome could be elicited in the rats throughout the 12 days of morphine pellet implantation.

5 The administration of 20 mg kg^{-1} morphine sulphate to anaesthetized rats did not decrease the contractions of the urinary bladder to repetitive stimulation of its motor nerves at 1 and 20 Hz.

Introduction

Many of the actions of morphine and its congeners are peripheral as opposed to the more familiar effects of these drugs on the central nervous system. Most notable perhaps is the spasmogenic action on smooth muscle of the uterus, gall bladder, bronchioles and the intestine (Burks & Long, 1967; Gillan & Pollock, 1980). Therapeutic doses of opioids also cause urinary retention. Early studies attributed this effect on micturition to excitatory actions of the opioids on the sphincter muscle of the bladder (Czapek & Wassermann, 1914; Ikoma, 1924) and also on the detrusor muscle (Duzen *et al.*, 1940; Winter, 1941). Either of these actions could be responsible for the impairment of voiding.

The micturition reflexes, integrated in the sacral spinal cord, enable sphincter relaxation and detrusor contraction to occur simultaneously. It appears likely that morphine, acting upon specific opioid receptors, interferes with these reflex pathways (Dray & Metsch, 1984). To this end it seemed reasonable to examine the action of morphine on micturition of conscious rats; the threshold to induce voiding may be measured repeatedly in rats made to void by water loading (Carpenter, 1981). Moreover, the efficacy of micturition can also be assessed by a determination of the urine volume remaining in the bladder when voiding is completed.

Also of concern in this study was the development of tolerance to the action of slow-release morphine on micturition. In mice and rats the antinociceptive action of slow-release morphine disappears rapidly despite a relatively high concentration of the drug in the blood; within 72 h after the slow-release morphine is administered the amount of morphine needed to obtund the tail-flick response increases 4 to 7 times that required in control animals (Cochin & Kornetsky, 1964; Way *et al.*, 1969; Cox *et al.*, 1975). Accordingly, the tail-flick and the voiding threshold were measured in rats during 12 days of pellet implantation.

Finally, since morphine depresses micturition, markedly increasing the volume threshold for micturition (MV) in the dependent state, an opposite effect on MV might occur in the withdrawn state. Thus, micturition by water-loaded rats was monitored following pellet removal and after opioid receptor antagonists were administered (Wei, 1973).

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Methods

Micturition was monitored in conscious rats for about 3 h after water was administered by stomach tube. Adult male rats weighing between 450–550 g were placed in metabolism cages following a standard water load equal to 5% of body weight. Urine was collected in a plastic beaker which was secured to a force transducer located beneath the outlet from the cage. As voiding occurred the volume passed was recorded on an oscillograph; the transducer which was coupled to a pen-writer by a carrier-wave amplifier, provided a linear chart displacement. Since the transducer output was calibrated, the volume collected in the beaker could be measured directly from the displacement (Carpenter, 1981).

A micturition threshold volume was obtained for each rat; the mean micturition volume (MV) resulted from the mean of 4 different trials. However, there was of course only one trial for each rat on a specific day following pellet implantation. When either morphine, naltrexone or naloxone were administered during an experiment the rat was removed from its cage and restrained while a subcutaneous injection was performed. All drugs were administered subcutaneously following the water load; however, the antagonists were given only after several voiding responses appeared on the recording. To avoid any possible development of tolerance, morphine was administered only once.

To measure the efficacy of micturition in the morphine-treated rats, measurements were made of the volume of urine which may have remained in the bladder when voiding was over. Most of the residual urine could be expressed by the application of external pressure to the bladder itself. After micturition appeared to be complete the animal was removed from the cage. While restraining the rat in one hand gentle pressure was applied to the bladder by the other. After the organ was localized between thumb and forefinger, urine was expressed, collected on absorbent paper and weighed.

Morphine base pellets containing 75 mg morphine (Gibson & Tingstadt, 1970) were implanted in rats after they were anaesthetized with diethyl ether and the dorsal lumbar hair removed. A 2 cm skin incision was made along the midline and after the subcutaneous fascia was separated by blunt dissection, one tablet was inserted in each side. The incision was closed by wound clips. In a second group of rats, 2 additional tablets were inserted on the following day.

To monitor spontaneous withdrawal, pellets were removed from a group of rats under diethyl ether anaesthesia on the 7th day of implantation. A stab incision was made above each pellet; these were clearly visible since they had become surrounded by granulomatous tissue. To assure complete removal of the material remaining within this area, it was repeatedly irrigated with saline.

Anaesthetized rats were used to identify any peripheral actions of morphine on the bladder musculature or at the junction between postganglionic motor nerve terminals and the muscle fibres. Adult male rats (500 g) were anaesthetized with a mixture of diallylbarbituric acid (80 mg kg^{-1}), ethyl carbamate (320 mg kg^{-1}), monoethylurea (320 mg kg^{-1}) and maintained on an animal warming board at 37°C. The pressure in the bladder was measured through a cannula inserted in the urethra, and connected to a P-37 Statham pressure transducer by polyethylene tubing. Bladder contractions, recorded as pressure elevations, were recorded on an ink-writing oscillograph. Both pelvic nerves were dissected free from the lateral surface of the prostate gland, placed on shielded platinum electrodes and surrounded by mineral oil. To obtain contractile responses of the bladder under optimal physiological conditions it was filled with 0.5 ml of saline.

Square-wave pulses 1 ms in duration and between 7–15 V intensity were applied at 1 and 20 Hz for 5 s; these stimulus trains elicited contractions of the bladder at intervals of 100 s. Morphine sulphate, which was administered intravenously, very often causes either ventilatory depression or apnoea in anaesthetized rats. Therefore the animals were routinely ventilated artificially by intermittent positive pressure (Bowen *et al.*, 1979).

Antinociception was measured by the radiant heat tail-flick test (D'Amour & Smith, 1941; Dewey *et al.*, 1970); rats were tested before and after administration of morphine. While the rat was restrained in an adjustable perspex cage, light from a 300 W projector was focused on the tail. The latency to removal of the tail from the stimulus was then recorded. If no response was manifested after 10 s the test was terminated.

Drugs

The following drugs were used: diallybarbituric acid (CIBA); diethyl ether (Mallinckrodt); ethyl carbamate (Fisher); morphine-base pellets (Gibson & Tingstadt, 1970) were obtained from a pharmaceutical supply company; morphine sulphate (Merck); naloxone and naltrexone hydrochloride (Endo).

Results

Micturition is dependent upon reflex pathways through the sacral cord and midbrain and is initiated when the bladder is sufficiently distended by the accumulation of urine. Intraluminal pressure is markedly elevated when the bladder contracts, urine being

discharged through the urethra after the sphincter muscles have relaxed (Garry *et al.*, 1959). In a rat undergoing a water diuresis the volume or threshold at which micturition is initiated appears to be fairly consistent. For the 3 rats shown in Figure 1 the volumes collected during 4 trials indicate that micturition is initiated by a narrow range of volumes in each rat. Moreover, the emptying process was virtually complete since very little urine could be manually expressed from the bladder when micturition was completed.

Water-loaded rats micturate at regular intervals, around 10 voiding responses were required to eliminate 80% of the water load. Elimination was linear during the 2 h required for the water to be excreted (Carpenter, 1981). To establish the micturition threshold for an individual rat the mean ± s.e.mean of around 40 responses was obtained after no less than 4 trials. The mean volume (MV) or micturition threshold in 43 untreated rats amounted to 2.6 ± 0.1 ml. In control animals the voiding trials were performed at intervals of 2–3 days.

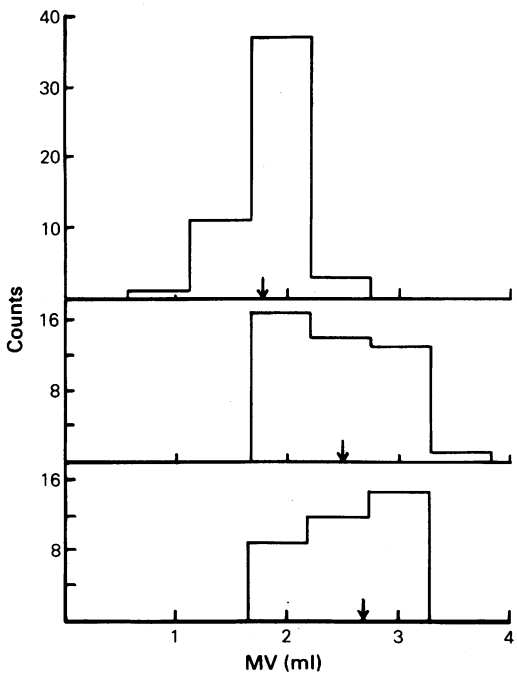

Figure 1 Pooled distribution frequency of the volumes of urine voided by 3 rats over a 2 h period which resulted from a water load equal to 5% of the animal's weight. The mean volume threshold for micturition (MV) resulting from all of the trials is shown by the arrow. Ordinate scale: the number of times voiding occurred within the volume range indicated on the abscissa scale in ml.

Voiding by water-loaded rats following subcutaneous injection of morphine sulphate

Morphine sulphate (5 mg kg^{-1}) suppressed the elimination of water in the loaded rats and increased the volume threshold to initiate voiding. Urine formation was decreased for at least an hour; the slopes of the recordings, which indicate the rate of urine formation by each rat, were reduced to less than half that of control rats.

A mean volume threshold (MV) of 2.5 ± 0.1 ml (s.e.mean) was obtained in a group of 16 rats before the administration of morphine sulphate. In all but one animal the MV of each rat was increased nearly 40% above its own control by morphine sulphate (5 mg kg^{-1}) and the group mean was increased to 3.4 ± 0.1 ml. Furthermore, in 8 of the rats urine was not actively expelled from the bladder at all by periodic voiding but was eliminated instead by leaking continuously through the urethra. Since the perineum was flooded with urine the rats appeared to be incontinent. However, very large volumes of urine were retained in the bladder and amounts much larger than control were recovered by manual pressure. Nevertheless, later during the trial once micturition had begun, emptying was complete; when voiding was over no significant amount of urine remained in the bladder.

Micturition in water-loaded rats following implantation of slow-release morphine pellets

In the first series, the micturition responses of 27 rats implanted with 2 morphine-base pellets were examined. The voiding patterns of these animals were recorded on the 3rd, 5th, 7th, 10th and 12th day after implantation. Although tolerance has been reported to develop rapidly to the antiduretic action of morphine (Inturrisi & Fujimoto, 1968; Huidobro, 1978) water was eliminated more slowly by the implanted rats for as long as 3 days after the pellets were inserted. However, by 5 days water elimination was nearly the same as that in the untreated rats.

A clear effect on the micturition threshold of 2 slow-release morphine pellets could be identified for as long as 12 days. In Figure 2 the closed circles indicate the group means of the rats that had been implanted with 2 pellets and are plotted at different times over a period of 12 days. The group means from day 3 (5.7 ± 0.3 ml) to day 7 (6.3 ± 0.4 ml) were more than twice the group mean of control or non-implanted rats (2.6 ± 0.14 ml) (Figure 3). After 5 days the mean threshold volume which initiated micturition in the implanted group (6.6 ± 0.3 ml) was significantly larger than the group mean at day 3 but was not different from the group mean at day 7. At day 10 the group mean (4.7 ± 0.3 ml) was 80% higher than control and at day

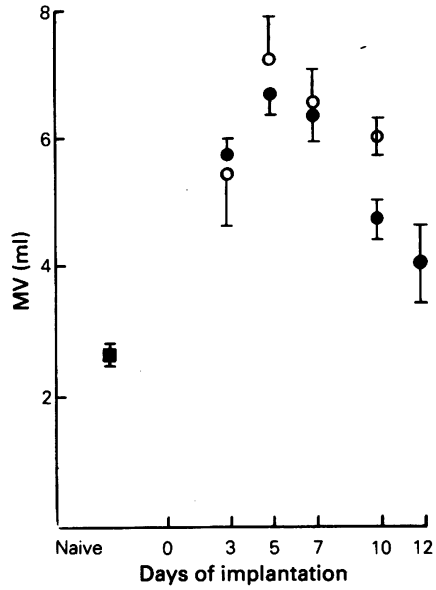

Figure 2 The action of slow release morphine on micturition in rats; the effect of implanted morphine base pellets on the mean voiding volume (MV) over a period of 12 days. (■) Group mean of 28 naive rats; (●) group mean of 27 rats implanted with 2 pellets for 10 days and 8 rats implanted with two pellets for 12 days; (○) group mean of the MVs from 16 rats implanted with 4 pellets for 10 days. Ordinate scale: MV in ml. Abscissa scale: days after implantation. Vertical lines show s.e.mean.

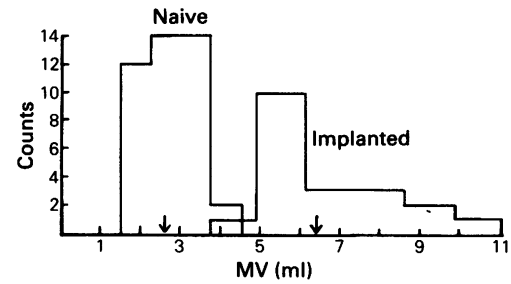

Figure 3 Pooled distribution frequency of the mean urine volumes (MV) voided over a 2 h period by naive ($n = 28$) and pellet (2)-implanted ($n = 27$) rats on day 7. A water load equal to 5% of the animal's weight was administered before each trial. The mean for each group is shown by the arrow. Ordinate scale: the number of times voiding occurred within the volume range indicated on the abscissa scale in ml.

12 the group mean was 50% above control (4.1 ± 0.6 l). The differences between the means for the control and the implanted rats at these intervals were significant for both, $P < 0.001$.

In the second series, 16 rats received 4 morphine pellets; their voiding responses were recorded on the 3rd, 5th, 7th and 10th day. The group means for these rats are shown by the open circles in Figure 2. With the exception of day 10, the group means of the rats given 4 pellets were not significantly different from the group means of the rats given 2 pellets. Voiding did not occur consistently in 8 of the animals after the water load; anuria, urinary retention and incontinence were manifest especially during trials on the 3rd and 5th day following implantation.

Micturition by water-loaded pellet-implanted rats following pellet removal

Excision of the pellets always resulted in a sizable reduction of the MVs by the following day. On day 7 when the pellets were removed from the rats given 2 or 4 pellets, the group means were twice those of naive controls, 6.4 ± 0.3 and 6.5 ± 0.5 ml, respectively (Figures 2 and 3). On the day following pellet removal (day 8) the group mean of the MVs from 10 rats implanted with 4 pellets was 1.10 ± 0.17 ml; the mean of 11 rats implanted with 2 pellets was 2.1 ± 0.14 ml, somewhat less than naive controls (2.6 ml). Thus, on the day following withdrawal of the rats from 2 pellets, the voiding threshold was restored to its normal setpoint. However, the withdrawal of the rats from 4 pellets resulted in a sizable reduction in the voiding threshold. On the 3rd–5th day following removal, both group means were the same as control. The bladder of the rats in either group was never distended after the pellets were removed and manual palpation revealed that it had emptied completely during micturition.

Micturition by water-loaded pellet-implanted rats during precipitated withdrawal

Opioid receptor antagonists were administered to 13 rats during their voiding trials on the 7th, 9th and 12th day after the implantation of 2 morphine-base pellets. The effects produced by naloxone ($0.8\ mg\ kg^{-1}$) or the longer acting naltrexone ($2\ mg\ kg^{-1}$) provided an indirect means to assess the action of the morphine released by the pellets throughout the 12 day period. Withdrawal signs such as wet dog shakes, chattering of the teeth and frequent passing of watery faeces were elicited shortly after antagonist administration (Way *et al.*, 1969; Wei, 1973; Ho *et al.*, 1979). Micturition occurred more frequently after the antagonists were administered and the MVs were reduced substantially from the controls (range 0.3 to 1.4 ml). During the trials of 7 animals the pen movements were too small to be accurately resolved; MVs were obtained by

dividing the volume collected by the number of discernible micturition responses. The MVs for these 7 rats were all less than 0.3 ml. The group mean for the other 6 animals amounted to 0.9 ± 0.2 ml. No differences were found between the MVs of rats withdrawn by either antagonist, from the 7th through to the 12th day. Manual palpation of the bladder in the acutely withdrawn rats did not reveal any distention. In 5 rats that had not been implanted with pellets the MVs were unchanged by either antagonist.

The action of morphine sulphate on nerve-induced bladder contractions in anaesthetized rats

Nerve-induced contractions of the bladder were elicited at stimulus rates of 1 and 20 Hz before and after administration of morphine sulphate. In this study morphine did not produce any excitatory effects on the bladder manifested as transient elevations in intraluminal pressure (Edmonds & Roth, 1920; Duzen *et al.*, 1940). The highest dose, 20 mg kg^{-1}, was 4 times the amount needed to obtund the nociceptive responses of conscious rats and 2 times the dose which produces ventilatory depression in anaesthetized rats.

Maximal contractile responses were produced during stimulation of both pelvic nerves at a stimulus rate of 20 Hz. In a series of 10 animals a mean pressure of 85 ± 5 cmH_2O developed during 5 s stimulus trains. Morphine sulphate (20 mg kg^{-1}) did not reduce the magnitude of these contractions 20 min after it was administered (Figure 4). However, it is most characteristic of morphine and other opioids to block nerve-induced responses in other autonomic organs at low stimulus rates (Paton, 1957; Henderson *et al.*, 1972; Hughes *et al.*, 1975). Therefore, responses were also elicited in the same animals by 1 Hz stimuli. The mean response to 1 Hz stimulus trains was 21 ± 2 cmH_2O and during repeated stimulation the responses were not diminished 20 min after the administration of morphine sulphate (20 mg kg^{-1}).

Antinociceptive effects in rats resulting from subcutaneous and slow release morphine

Each of the 8 naive rats responded to the nociceptive stimulus; within 2–3 s (mean = 2.8) after the radiant heat source was applied there was a fast movement of the tail away from the stimulus. This response was obtunded 40 min after morphine sulphate (5 mg kg^{-1}) was administered; the latency of the response increased to 8 s or more. To avoid injury the stimulus was discontinued after 10 s. Six hours after 2 morphine-base pellets were implanted the response of 8 animals to the heat source was also obtunded; in only one of the rats was a weak tail-flick produced within 10 s. On the 3rd day following implantation the mean latency was 4.3 s suggesting that the rats had become tolerant to the analgesic actions of the slow release morphine. On the 5th day the latency for the tail-flick response was the same as control.

Discussion

After 5 mg kg^{-1} morphine sulphate was administered to the rats their responsiveness to non-nociceptive stimuli as well as their state of awareness appeared to be undiminished; the animals quickly assumed an upright posture after being released from the supine position. Moreover, studies by Babbini & Davis (1972) suggest that the spontaneous locomotor activity of rats actually increased after this dose. Nevertheless, the depressant effect of morphine on spontaneous behaviour produced by the implantation of 4 pellets is quite apparent until day 3.

Changes in micturition in conscious water-loaded rats are produced by morphine over a dose range similar to that suppressing the tail response to a nociceptive stimulus. In the initial study of the tail-flick by D'Amour & Smith (1941), a dose of 12 mg kg^{-1} of morphine sulphate was needed to eliminate the response in 92% of the rats. Surprisingly, none was protected by 6 mg kg^{-1} morphine sulphate. An even larger dose (20 mg kg^{-1}) was needed to obtund the responses of rats on a hot plate (Cochin & Kornetsky, 1964). In mice, the analgesic dose which eliminated the tail flick in 50% of the animals was 10 mg kg^{-1} (Way *et al.*, 1969; Dewey *et al.*, 1970). Morphine antidiuresis in rats occurs in the same dose range as urinary retention, namely 4–8 mg kg^{-1} (Inturrisi & Fujimoto, 1968; Huidobro, 1978).

There is some similarity between the action of morphine on micturition and on defacation; urinary retention and constipation usually occur simultaneously during opioid therapy. Although therapeutic doses of morphine may be spasmogenic on the large intestine and the dog bladder (Edmonds & Roth, 1920; Duzen *et al.*, 1940; Winter, 1941) no similar effect was found on the rat bladder (Figure 4). The spasmogenic action of the opioids on other multiunit smooth muscles such as the gall bladder and the bronchioles, is probably not at all similar to their action on the large intestine. Their effect on the latter organ is more complex owing perhaps to intramural or enteric neurones and to the variety of neurotransmitters in that organ (Burks & Long, 1967; Gillan & Pollock, 1980).

The depressant effect of morphine on micturition must be mediated centrally (Dray & Metsch, 1984). Contrary to the finding of Hukovic *et al.* (1965), junctional transmission in the bladder was not impaired by morphine at a dose estimated to be twice that employed by these authors. This stands in contrast to the marked depressant effect of morphine on nerve-

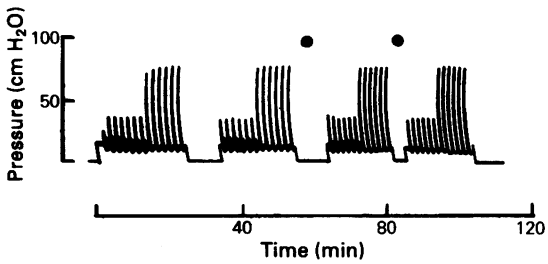

Figure 4 Recording of the intraluminal pressure in the urinary bladder of an anaesthetized rat during intermittent stimulation of the pelvic nerves. The minimum and maximum contractile responses which appear on the trace were produced by 1 and 20 Hz stimulus trains, respectively. (●) Indicates administration of morphine sulphate, 10 mg kg^{-1}, i.v.

induced responses of the mouse vas deferens (Henderson *et al.*, 1972; Hughes *et al.*, 1975), the guinea-pig ileum (Paton, 1957) and the rat parotid gland (Bowen & Carpenter, 1979), at low stimulus rates.

Because of the viscoelastic behaviour of smooth muscle the bladder itself will accommodate a larger volume during a low rate of filling. Therefore, the reduced urine formation following morphine might cause the MV to increase. However, in conscious rats, when the bladder is distended more rapidly the voiding threshold actually became larger; during a water diuresis the higher filling rate resulted in a larger rather than a smaller MV (Carpenter, 1981). The action of morphine on urine production alone cannot explain why voiding failed to occur at all in 8 of the treated rats unless the drug had elevated the voiding threshold.

In a number of animal experiments in which morphine was observed to cause urinary retention, an excessively active urethral sphincter appeared to be responsible (Czapek & Wassermann, 1914; Ikoma, 1924); an action of the drug on the sacral cord was believed to have paralyzed inhibitory influences of the pelvic nerves on the sphincter. The present study could not implicate any sphincter dysfunction as causing retention in morphine-treated rats. While sphincter-detrusor incoordination is very often the cause of incomplete voiding in other conditions, no urine remained following micturition in the treated animals; after the threshold was elevated 2–3 fold the bladder was always empty after voiding was over.

There was a ceiling dose for slow release morphine on the micturition threshold; the implantation of 4 pellets elevated the mean MV to about the same extent as 2 pellets. Of course the extent to which MV could increase would be physically limited by the distensibility of the bladder. However, by day 10 the effect of morphine from 4 pellets was significantly greater than the effect from 2 pellets. At this time, morphine release from the pellets has begun to decline and its concentration in the plasma is correspondingly less (Bowen *et al.*, 1979). Any tendency for $MV_{implant}/MV_{naive}$ to decrease after day 10 most probably was due to a decreasing concentration of morphine in the plasma (Bowen *et al.*, 1979) and not to the development of tolerance. As this and other studies have shown, tolerance to the antinociceptive action of morphine develops rapidly in mice and rats (Cochin & Kornetsky, 1964; Way *et al.*, 1969; Cox *et al.*, 1975; Lange *et al.*, 1983). Three days after morphine was administered continuously to mice or rats the initial antinociceptive dose was no longer effective. In contrast, throughout 10 days of slow-release morphine administration, the volume threshold for micturition in rats remained elevated.

Ventilation in anaesthetized rats also did not become tolerant to slow-release morphine from implanted pellets (Bowen *et al.*, 1979). After 6 days, the pulmonary minute volume of implanted rats was only 42% of naive rats. The medullary neurones which maintain spontaneous ventilation in the anaesthetized pellet-implanted rat were depressed by morphine in proportion to its plasma concentration. However, as a consequence of sustained release of morphine by the pellets, the neuronal pathways responsible for ventilation were more tolerant to intravenous morphine.

Tolerance and dependence may develop in parallel during the prolonged administration of opioids. In this study of micturition in rats, there seemed to be no direct relationship between dependence and the development of tolerance. After the action of the slow release morphine is terminated by pellet removal or by an opioid receptor antagonist, the voiding threshold was always decreased. The conversion by naloxone of a high micturition threshold to one substantially less than control is also an example of withdrawal; the neural pathways responsible for micturition were morphine-dependent.

During an extended exposure of neurones to morphine certain adaptive changes occur in their sensitivity to opioids and probably account for the development of phyisological or homeostatic tolerance; an equilibrium between morphine in the plasma and a specific population of neurones may be limited (Lange *et al.*, 1983). For the neuronal systems responsible for ventilation and micturition in rats, there is apparently no such limitation in the action of morphine on these sites.

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