Br. J. Pharmac. (1979), **65**, 457–463

VENTILATORY DEPRESSION IN NAIVE AND TOLERANT RATS IN RELATION TO PLASMA MORPHINE CONCENTRATION

S.R. BOWEN, F.G. CARPENTER & J.G. SOWELL

Department of Pharmacology, University of Alabama in Birmingham, Birmingham, Alabama 35294, U.S.A.

1 The disappearance of morphine from specially formulated pellets containing 75 mg morphine base was measured for 10 days after they were implanted into adult rats; the morphine content decreased at a rate of 5 mg pellet daily.

2 From the 2nd to the 6th day of implantation the plasma morphine concentration increased but by the 10th day had declined to only one half the concentration found on day 6.

3 Six and 24 h after the pellets were removed from 6 day implanted animals the plasma concentration of morphine amounted to only one quarter to one sixth of the amount in the plasma, respectively, of animals with pellets intact.

4 The pulmonary minute volume of naive and implanted rats was depressed by morphine in proportion to the plasma morphine concentration. Less depression was produced by intravenous morphine in the implanted rats than in the naive animals; the greater morphine tolerance displayed by the implanted animals could be shown by the third day of implantation and appeared to be maintained to the 10th day.

5 The pulmonary minute volume of implanted rats on the 6th day was much less than the pulmonary minute volume of naive rats. Six and 24 h after the pellets were removed the pulmonary minute volume increased as the plasma morphine concentration decreased.

6 The effects on the pulmonary minute volume produced by the slow release of morphine from the implanted pellets was not changed by the development of tolerance while the effects of morphine produced by rapid injection were diminished by the development of tolerance; the different effects of morphine are accordingly linked to the mode of administration.

7 We conclude that the action of morphine on the pulmonary minute volume in tolerant rats following rapid injection is fundamentally different from its action following its slow release from implanted pellets, possibly due to differences in access to an undefined neuronal site.

Introduction

The use of morphine in the treatment of pain is invariably limited by its depressant effect on ventilation. Many studies have indicated that therapeutic amounts of nearly all narcotic analgesic drugs will significantly decrease the pulmonary minute volume presumably by lowering the sensitivity of the respiratory centre to CO_2 (Eckenhoff & Oech, 1960). During the continued use of morphine human subjects become tolerant to the ventilatory depression by morphine and other opiates. With the development of tolerance the lethal dose of morphine is greatly increased; however, a dose always exists that is capable of producing death from respiratory depression in tolerant individuals (Jaffe & Martin, 1975).

The relation between tolerance and the morphine concentration in plasma can be systematically examined in experimental animals (Goldstein & Schulz, 1973). In this study measurements of the pulmonary minute volume in anaesthetized rats were made before and after morphine. Other animals were made tolerant to morphine by the implantation of specifically formulated pellets of morphine base (Way, Loh & Shen, 1969; Gibson & Tingstad, 1970.) In parallel studies, the morphine concentration in the plasma was determined at various intervals after the pellets were implanted and also following their removal. Ventilation in the conscious rat is not particularly sensitive or susceptible to morphine; the animals survive intravenous doses as large as 100 mg/kg without serious respiratory difficulty (Martin, Wikler, Eades & Pescor, 1963). In contrast, rats anaesthetized with Dial-urethane and maintaining quite adequate ventilation were markedly depressed by doses in the range of 2 to 5 mg/kg (Bowen & Carpenter, 1979).

Because of the marked effect of morphine on respiration in anaesthetized rats their minute volume was

0007-1188/79/030457-07 $01.00

chosen as a means of estimating tolerance to morphine in pellet-implanted animals. Our findings suggest that two quite different effects are exerted by morphine on the pulmonary minute volume which depend on the mode of administration; tolerance was manifest following the rapid intravenous injection of the alkaloid but not to the morphine slowly released from implanted pellets.

Methods

Male Sprague-Dawley rats weighing between 350 to 450 g were anaesthetized with Dial-urethane, 0.7 ml/kg (70 mg/kg of diallylbarbituric acid and 280 mg/kg ethyl carbamate) by intraperitoneal injection. A stainless steel cannula, inserted and tied into the trachea, allowed frequent aspiration of any secretions which accumulated during the procedures. Body temperature was maintained at 37°C with a warming board. Morphine and naloxone were administered into the femoral vein with a 27 G needle by rapid injection.

The pulmonary minute volume (PMV) was measured in anaesthetized animals by collecting the expired air in a 2.5 litre spirometer over a 10 min period (De Haven & Carpenter, 1964). The displacement of the cylinder was recorded at 1 min intervals. The PMV was measured only when the animal's body temperature had stabilized at 37°C and while the animal was breathing room air. In the figures, PMV (or the pulmonary minute ventilation) is expressed at BTPS. Control measurements were made on each animal repeatedly until a uniform minute volume was established (Bowen & Carpenter, 1979). With this method, the PMV measured over a 10 min period was always found to be linear and consistent from one animal to another. This suggested that both the spirometer and valve system did not place an unusual burden on the animal's ventilatory system. In anaesthetized rats of approximately 400 g wt. and untreated with morphine, we obtained a mean rate of ventilation amounting to 145 ± 4 ml/min BTPS. From the empirical formula of Guyton (1947) a figure was obtained of 156 ml/min for animals of this body weight.

Pellet implantation

Each pellet contained 75 mg of morphine base together with various binding agents (Gibson & Tingstad, 1970). The animals were anaesthetized with diethyl ether and 2 pellets were implanted subcutaneously in the dorsal flank; 24 h later 2 additional pellets were implanted. If as many as 4 pellets were implanted at the same time, a significant mortality resulted. In most animals the 4 pellets were removed after 3, 6 or 10 days under diethylether anaesthesia and either 6 or 24 h allowed to elapse before the experiment was begun. In some animals the experiments were conducted with the pellets intact.

Naloxone when given to tolerant animals produces a number of somatic effects which surprisingly are manifested in an anaesthetized restrained animal. Of immediate concern was the apparent arousal of the animal. This was suggested by the production of 'wet dog shakes' after the injection had been made. It should be mentioned in this connection that naloxone when given in the same amount to anaesthetized naive animals produced little effect on the minute volume.

Our implanted rats did not display diarrhoea or weight loss as was described by Johnson, Westfall, Howard & Fleming (1978) in the guinea-pig; these symptoms appeared in the rats only after the pellets had been removed for 24 hours.

Morphine content of pellets and plasma

The morphine content of pellets and its concentration in rat plasma was measured by a modification of the method described by Kupferberg, Burkhalter & Way (1964). Morphine base was extracted from the pellets in 500 ml 0.01 M HCl. One ml of 1:100 dilution was added to an equal volume of 0.5 M Tris buffer (pH = 8.5) and mixed with 0.1 ml of a 1:10 dilution of potassium-ferric-ferrocyanide reagent (57.7 mg potassium ferricyanide + 5 mg potassium ferrocyanide dissolved in 100 ml distilled water). This solution was read after 10 min in a Turner 430 spectrofluorometer; fluorescence was measured during 250 nm excitation and 440 nm emission. Whole blood was collected by exsanguination at various stages of implantation and/or after morphine was administered intravenously to the animals. Three ml of plasma and 0.5 ml of 0.5 M Tris buffer was mixed by an oscillating shaker for 15 min with a mixture of 90% chloroform-10% butanol. After centrifugation, 7.5 ml of the chloroform-butanol was recovered and mixed with 1.2 ml 0.01 M HCl on the oscillating shaker for 15 min; 1 ml of the aqueous phase was then mixed with 1 ml of the Tris buffer and 0.1 ml of the CN^- reagent. After 10 min the fluorescence at 440 nm during excitation at 250 nm was read after transferring the solution to quartz cuvettes. The recovery of known amounts of morphine added to rat plasma amounted to 70%. The concentration of morphine in the plasma and the content of morphine in the pellets is expressed as the base form.

Drugs and reagents

The following drugs and reagents were used: chloroform and diethylether (Mallinckrodt); diallylbarbi-

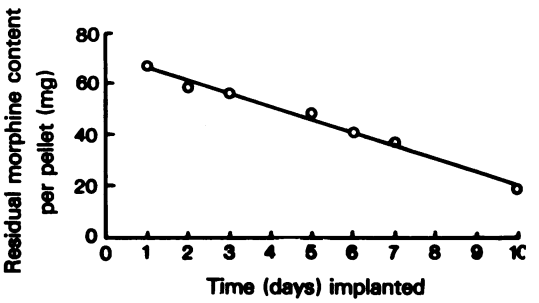

Figure 1 The morphine content of pellets at different intervals after they were implanted in adult male rats; (O) the mean content of approximately 15 pellets. Ordinate scale: mg of morphine base. Abscissa scale: time in days.

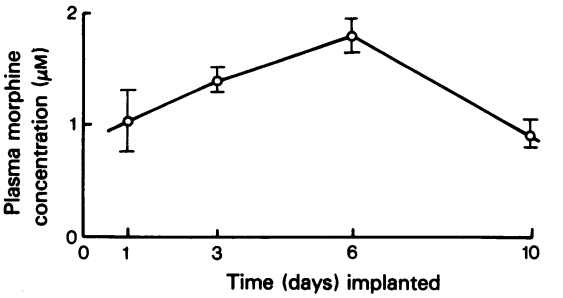

Figure 2 The mean concentration of morphine base in the plasma of adult male rats (O) measured at different intervals following the implantation of 4 morphine base pellets. At day 1 the animals had received only two pellets while thereafter each rat had received four pellets. Ordinate scale: plasma concentration of morphine base in μM. Abscissa scale: time in days. The s.e. mean is shown by vertical lines.

turic acid (CIBA); ethyl carbamate (Fisher); morphine sulphate (Merck); *n*-butanol (Baker); naloxone hydrochloride (Endo); trishydroxymethyl-aminomethane (Calbiochem).

Results

The disappearance of morphine from morphine-base pellets

Morphine pellets when implanted in rats and mice have been shown to produce a tolerance to morphine as well as a physical dependence on morphine in a short period of time (Maggiolo & Huidobro, 1961; Way, Loh & Shen, 1969). We implanted 4 such pellets in 67 rats and at the appropriate interval as shown in Figure 1 the pellets were removed from the animals and analyzed. The results are expressed as the mean of approximately 15 pellets. Throughout 10 days of implantation the morphine content was reduced linearly; a single pellet released an average of 5 mg of morphine base per day. Accordingly, the implanted animals received a constant daily dose of 20 mg of morphine from the four pellets. A regression analysis of all the data revealed a correlation coefficient of 0.92 for the best fitting straight line.

The relation between the plasma morphine concentration and the duration of pellet implantation

Plasma samples were analyzed for morphine content at various stages of implantation (Kupferberg *et al.*, 1964; Goldstein & Schultz, 1972; Berkowitz, Cerreta & Spector, 1974). Figure 2 shows that the concentration of morphine in plasma (Mp) ranged between 1 and 1.8 μM throughout the 10 day implantation period. Since only 2 pellets could be implanted initially the Mp for day 1 in Figure 2 is less than the amount which would have been expected had the rat received 4 pellets. However, on days 3, 6 and 10 the Mp resulted from the presence of 4 pellets in the animals. The mean concentration on day 1 was not different from day 3 or day 10. All other values were significantly different at the $P < 0.05$ level. Accordingly, the plasma morphine concentration increased from the 3rd to the 6th day at which time the maximum concentration was obtained. By the tenth day the concentration of morphine in the plasma had declined to values similar to day 1 which resulted from the implantation of only 2 pellets. The values in Figure 2 represent corrected values for 70% recovery of morphine from rat plasma.

The action of morphine on the pulmonary minute volume (PMV) in naive animals

The PMV was measured over a 10 min period in anaesthetized adult male rats maintained at a constant temperature of 37 ± 1°C; the mean minute volume measured in 19 animals amounted to 145 ± 4 ml/min BTPS. This figure is in agreement with previous reports by Guyton (1947), Masland & Yamamoto (1962) and Bartlett & Tenney (1963).

The mean PMV in non-implanted and in 3, 6 and 10 day pellet implanted animals is indicated in Figure 3 by the height of the columns. In the anaesthetized rat the administration of morphine depressed the minute volume in direct proportion to the dose. In Figure 3a the effect of morphine on the PMV of 8 naive rats is shown after doses of 2 and 5 mg/kg were administered intravenously; the mean PMV was depressed substantially by morphine. As shown by

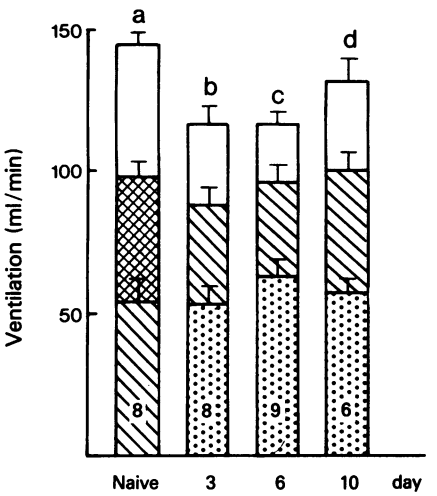

Figure 3 The action of morphine on the pulmonary minute volume (PMV) of anaesthetized rats. Column (a): the mean PMV of naive rats before, (upper), and after 2 mg/kg morphine (middle), and after 5 mg/kg morphine (lower) was administered intravenously. Columns (b), (c) and (d): the mean PMV of rats implanted with morphine for 3, 6, and 10 days respectively before (upper), after 5 mg/kg morphine (middle), and after 20 mg/kg morphine (lower), was administered intravenously. The s.e. mean is indicated by the vertical lines; *n* is shown within each column. Vertical scale: pulmonary minute volume in ml/min. Numbers along base line; duration of pellet implantation in days.

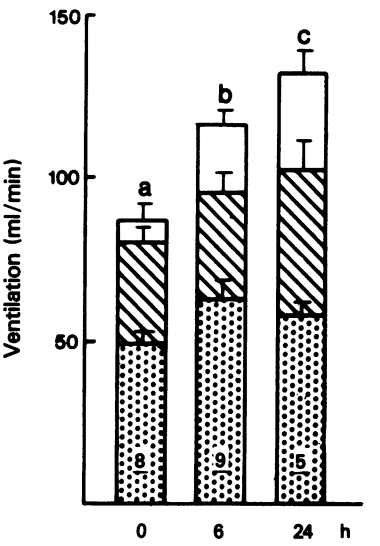

Figure 4 The action of morphine on the pulmonary minute volume (PMV) of rats implanted with morphine pellets for 6 days. Column (a): the mean PMV of rats in which the pellets were intact (upper), after 5 mg/kg morphine (middle) and after 20 mg/kg morphine (lower) was administered intravenously. Columns (b), (c): the mean PMV of rats in which the pellets had been removed for 6 and 24 h respectively (upper), after 5 mg/kg morphine (middle) and after 20 mg/kg morphine (lower) was administered intravenously. The s.e. mean is indicated by the vertical lines; *n* is shown within each column. Vertical scale: pulmonary minute volume in ml/min. Numbers along base line: duration of pellet removal in hours.

column (a) of Figure 3 the depression of the mean PMV amounted to 32% after the administration of 2 mg/kg while a dose of 5 mg/kg resulted in ventilatory arrest or apnoea in 9 out of 16 animals. The column indicating the effect of 5 mg/kg morphine represents only those animals that survived this dose. Accordingly, the depression of the mean PMV to 55 ml/min after 5 mg/kg morphine, as expressed in Figure 3, is somewhat misleading since only 9 rats are represented of the 16 animals that were used. An analysis of variance showed the minute ventilation values were significantly different for each dose of morphine that was studied.

The action of morphine on the pulmonary minute volume of implanted animals

The effect of graded doses of morphine on the mean PMV of anaesthetized rats was examined at various stages of pellet implantation (Figure 3). A single morphine pellet identical to those used in this study produce a maximum physical dependence and tolerance in Swiss-Webster mice on the third day of implantation (Way *et al.* 1969). The data shown in Figure 3 were obtained 6 h after the pellets were removed. In 23 implanted animals (Figure 3), the PMV was not as depressed by intravenously administered morphine as in the naive animals. In the control animals, 5 mg/kg morphine resulted in 63% depression of the PMV in those animals that survived (Figure 3). In contrast, the PMV of the pellet-implanted rats was depressed by only 22%. Moreover, the implanted animals were able to tolerate doses of morphine as high as 20 mg/kg (Figure 3) without apnoea.

Tolerance to morphine as measured by its effect on ventilation appeared to be well developed by the third day of implantation. Furthermore, the effects of 5 and 20 mg/kg morphine sulphate on the 3rd, 6th and 10th day were the same; there was no significant difference between the 3 groups. All of the implanted animals were able to tolerate 20 mg/kg morphine and this tolerance was maintained through the tenth day; no apnoea was observed in the implanted

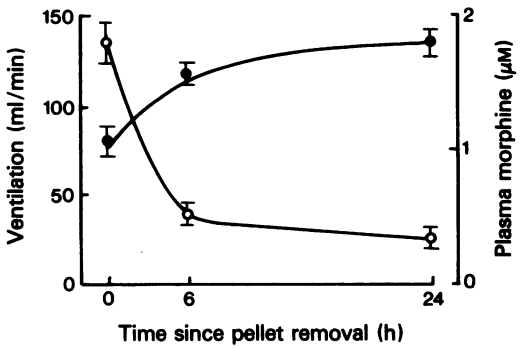

Figure 5 The pulmonary minute volume (●) and plasma morphine concentration (○) of pellet-implanted rats before and after their removal from the animals. The initial values in 6 days rats containing the pellets are indicated above the zero on the abscissa scale while values obtained at 6 h and at 24 h following the removal of the pellets are indicated at the appropriate intervals. Left ordinate scale: pulmonary minute volume in ml/min. Right ordinate scale: plasma morphine concentration in μM. Abscissa scale: time in hours since the pellets were removed. The s.e. mean is shown by the vertical lines.

animals following the 20 mg/kg dose of morphine. It was surprising to find the means of the initial PMV on the third and sixth day of implantation were less than the mean of the non-implanted animals (Figure 3). An analysis of variance of each group showed that the mean PMV was depressed significantly following acute morphine doses.

Although the release of morphine base from the pellets was responsible for the development of tolerance in the implanted animals, their PMV was nevertheless depressed as a consequence of the morphine that was slowly released by the implanted pellets. This was shown by comparison of the PMV of anaesthetized rats before (0) and 6 and 24 h after the pellets had been removed (Figure 4). In 8 rats in which the pellets had remained (0), the mean PMV was only 84 ml/min which is considerably less than the PMV of naive animals (145 ml/min, Figure 3). Six hours after the pellets were removed from 9 of the implanted animals the PMV increased to 117 ml/min (6). After the pellets had been removed for 24 h the mean ventilation in 5 animals increased to 133 ml/min however the difference between the 6 and 24 h group was not significant (Figure 4).

The effect of 5 and 20 mg/kg morphine on the minute volume is also indicated for each of the three groups by the shaded areas of the columns (Figure 4). No difference could be shown between the means of the 6 and 24 h group after 5 and after 20 mg/kg morphine had been administered. However, differences within each group were significant ($P < 0.05$) except for the initial PMV and the PMV following 5 mg morphine in animals in which the pellets were not removed.

The plasma concentration of morphine in implanted animals after excision of the morphine pellets

In Figure 5 are shown the plasma morphine concentrations (Mp) of 6 day-implanted rats, in which the pellets had either remained intact or had been removed for 6 h or for 24 hours. The removal of the pellets from the implanted animals on day 6 resulted in a significant reduction in Mp (Figure 5). Before the pellets were removed the mean Mp amounted to 1.8 μM while 6 h after the pellets were removed it decreased 66% to only 0.53 μM. After 24 h the mean Mp (0.34 μM) was less than the 6 h mean but the difference between the 6 and 24 h means was not significant.

Also shown in Figure 5 is the mean of the initial PMV of the 3 groups of animals from Figure 4. There is a clear inverse relation between the two curves; ventilation increased markedly as the Mp diminished following pellet removal. The initial PMV of the implanted animals at 6 days is thus an indirect function of the Mp; 6 h after the pellets were removed the mean PMV in this group of animals increased with a concomitant decrease in Mp. Moreover, a further increase in the initial PMV of the animals occurred 24 h after the pellets were excised and during this time the Mp had decreased even further.

In Figure 6 PMV of naive and 6 day-implanted animals are plotted against the plasma morphine concentration (Mp). Figure 6a shows data obtained following the administration of 2 and 5 mg/kg morphine to naive rats (0). Also shown is the relation between PMV and Mp in implanted animals before (▲), 6 h (△) and 24 h (●) after removal of the morphine pellets. There was no apparent tolerance to morphine in the implanted animals before (▲) or after (△), (●) pellet removal. The points from the pellet implanted animals lie on the same curve as the points from the naive animals following the administration of 2 and 5 mg/kg morphine. Accordingly, the PMV of both naive and implanted animals was depressed in direct proportion to the Mp (slope = 24.7 ml min^{-1} μM^{-1}).

However, the implanted group was tolerant to morphine as may be established from the data plotted in Figure 6b which shows the extent of the pulmonary depression produced by intravenous morphine in the implanted animals. For example, after 20 mg/kg morphine, the Mp in rats 24 h after pellet removal amounted to 21.8 μM (●) but the depression of the PMV produced by this dose did not exceed that produced by 5 mg/kg morphine in the naive animals. In animals with pellets intact, a dose of 20 mg/kg

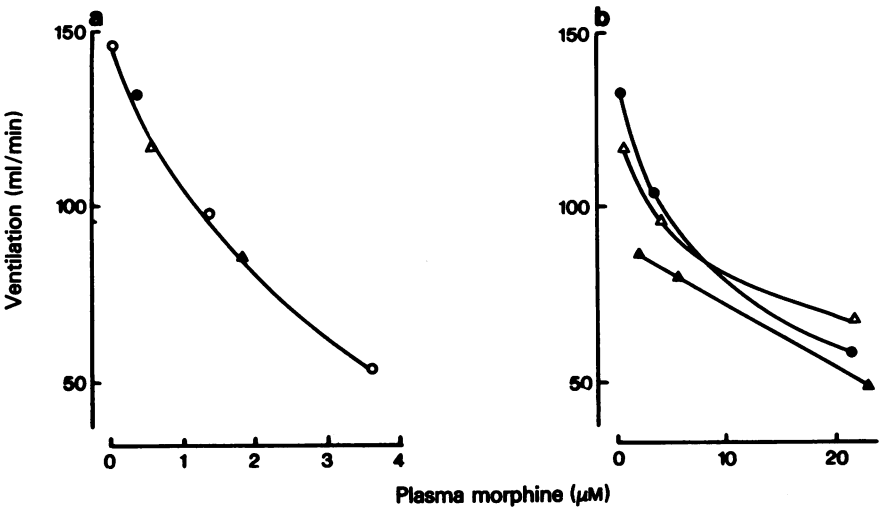

Figure 6 The relation between the mean pulmonary minute volume and the mean plasma morphine concentration in naive and morphine pellet-implanted rats. (a) (○): Naive rats before and after 2 mg/kg morphine and after 5 mg/kg morphine were administered intravenously; (●): 6 day-implanted rats, pellets removed for 24 h; (△): 6 day-implanted rats, pellets removed for 6 h; (▲): 6 day-implanted rats, pellets intact. (b) Six day-implanted rats before and after 5 mg/kg morphine and after 20 mg/kg morphine was administered intravenously. (●): Pellets removed for 24 h; (△): pellets removed for 6 h; (▲): pellets intact. Ordinate scale: pulmonary minute volume in ml/min. Abscissa scale: plasma morphine concentration in μM. Each point represents the mean result from at least 6 animals.

morphine extended the Mp to 23 μM (▲). Six h after the pellets were excised (△), the Mp was increased to 21 μM following the 20 mg/kg dose but again the effect of the PMV was no greater than that produced by 5 mg/kg morphine in a naive animal. When only 5 mg/kg morphine was administered to the implanted animals the Mp increased to approximately 4 μM but the PMV was never depressed by more than 21% (Figure 6b). The slope function for the group with intact pellets amounted to 1.8 ml min^{-1} μM^{-1}. Accordingly, the relation between PMV and Mp did differ in the implanted animals with respect to the source of the morphine. The rats did not appear to be tolerant to the slowly administered morphine released by the pellets (Figure 6a). In contrast the implanted animals were tolerant to rapid intravenous administration of morphine (Figure 6b).

Discussion

It is most unlikely that acute doses of morphine in the range 2 to 20 mg/kg would alter the PMV of unanaesthetized rats; the depressant action of morphine in this study is dependent upon the anaesthetic. In decerebrate cats, doses of morphine in the range 2 to 20 mg/kg, diminish respiratory rate and minute volume significantly from control (Martin & Eisenman, 1962; Florez, Armijo & Delgado, 1972). However, mature unanaesthetized rats are much less susceptible to morphine; doses amounting to 100 mg/kg produce little significant effect on the respiratory rate (Martin *et al.*, 1963). In fact doses as high as 200 mg/kg do not produce signs of neurological depression at all but instead elicit convulsive seizures (Sloan, Brooks, Eisenman & Martin, 1963). The tolerance displayed by our implanted rats to the depressant action of morphine on the PMV is about the same as that found in other studies where the same number of pellets were implanted in animals of about the same weight. For example, ileal-myenteric plexus strips became more tolerant to the inhibitory action of morphine on electrically induced twitch responses after the donor animals were implanted with 4 morphine-base pellets similar to the ones used in our study. These preparations, taken from guinea-pigs, required from 4.5 to 7 times as much morphine in the bathing medium as control preparations taken from naive animals (Goldstein & Schulz, 1973; Johnson, Westfall, Howard & Fleming, 1978).

In both naive and implanted animals the PMV was depressed in direct proportion to the Mp in the range 0 to 4 μM; 6 days after implantation the morphine released by the pellets produced the same degree of

ventilatory depression as acutely administered morphine did in the naive animals (Figure 6a). For example, after 6 days of implantation the release of morphine from the pellets resulted in a Mp of 1.8 ± 0.15 μM while the PMV of the 6 day animals with intact pellets was 84 ml or 58% of control. In naive animals, the administration of 2 mg/kg of morphine by rapid injection resulted in Mp of 1.3 μM and a PMV of 97 ml or 67% of control after this dose of morphine. Figure 6a indicates that the relation between Mp and PMV in both the implanted and the non-implanted animals is the same, despite the tolerance to morphine of the former group of animals. Accordingly, the slow release of 20 mg of morphine from the pellets over a period of 24 h maintains the plasma concentration at a level equal to that achieved by the rapid intravenous injection of 1.0 mg. Moreover throughout the 6 day period the slow release has maintained a uniform concentration of morphine in the plasma.

Within 6 and 24 h after the pellets were removed the PMV increased as a consequence of the lower Mp. Nevertheless under these conditions the animals tolerated intravenous doses of morphine that would have produced ventilatory arrest or apnoea in non-implanted animals. Apparently the neuronal pathways concerned with the production of spontaneous ventilation in anaesthetized rats did not become tolerant to the Mp that was maintained by the pellets throughout the 6 days of implantation. We believe that the prolonged release of morphine may enable an equilibrium to be established between the plasma and the neuronal site which governs the ventilation. In contrast, a rapid injection of morphine may not gain access to this site because of its short half-life (Berkowitz *et al.*, 1974) and its action on the neural site may thus be limited (Way & Adler, 1960).

References

BARTLETT, D., JR. & TENNEY, S.M. (1963). Tissue gas tensions in experimental anemia. *J. appl. Physiol.*, **18**, 734–738.

BERKOWITZ, B.A., CERRETA, K.V. & SPECTOR, S. (1974). The influence of physiologic and pharmacologic factors on the disposition of morphine as determined by radioimmunoassay. *J. Pharmac. exp. Ther.*, **191**, 527–534.

BOWEN, S.R. & CARPENTER, F.G. (1979). Morphine depression and tolerance of nerve-induced parotid secretion. *Br. J. Pharmac.*, **65**, 7–13.

DEHAVEN, K.E. & CARPENTER, F.G. (1964). Interaction between CO_2 and citrate ions in medullary reticular formation. *Am. J. Physiol.*, **207**, 298–302.

ECKENHOFF, J.E. & OECH, S.R. (1960). The effects of narcotics and antagonists upon respiration and circulation in man: A review. *Clin. Pharmac. Ther.* **1**, 483–524.

FLOREZ, J., ARMIJO, J.A. & DELGADO, G. (1972). Characterization of the acute tolerance and dependence to morphine-induced respiratory depression in decerebrate cats. *Rev. Espan. fisiol.*, **28**, 167–173.

GIBSON, R.D. & TINGSTAD, J.E. (1970). Formulation of a morphine implantation pellet suitable for tolerance-physical dependence studies in mice. *J. Pharm. Sci.*, **59**, 426–427.

GOLDSTEIN, A. & SCHULZ. R. (1973). Morphine-tolerant longitudinal muscle strip from guinea-pig ileum. *Br. J. Pharmac.*, **48**, 655–666.

GUYTON, A.C. (1947). Measurement of the respiratory volumes of laboratory animals. *Am. J. Physiol.*, **150**, 70–77.

JAFFE, J.H. & MARTIN, W.R. (1975). Narcotic analgesics and antagonists. In *The Pharmacological Basis of Therapeutics*, 5th edition. ed. Goodman, L. S. & Gilman. A. pp. 245–283. New York: Macmillan.

JOHNSON, S.M., WESTFALL, D.P., HOWARD, S.A. & FLEMING, W.W. (1978). Sensitivities of the isolated smooth muscle-myenteric plexus and hypogastric nerve—vas deferens of the guinea pig after chronic morphine pellet implantation. *J. Pharmac. exp. Ther.*, **204**, 54–66.

KUPFERBERG, H., BURKHALTER, A. & WAY, E.L. (1964). A sensitive fluorometric assay for morphine in plasma and brain. *J. Pharmac. exp. Ther.*, **145**, 247–251.

MAGGIOLO, C. & HUIDOBRO, F. (1961). Administration of pellets of morphine to mice; abstinence syndrome. *Acta physiol. latinoam.*, **11**, 70–78.

MARTIN, W.R. & EISENMAN, A.J. (1962). Interactions between nalorphine and morphine in the decerebrate cat. *J. Pharmac. exp. Ther.*, **138**, 113–119.

MARTIN, W.R., WIKLER, A., EADES, C.G. & PESCOR, F.T. (1963). Tolerance to and physical dependence on morphine in rats. *Psychopharmacologia*, **4**, 247–260.

MASLAND, W.S. & YAMAMOTO, W.S. (1962). Abolition of ventilatory response to inhaled CO_2 by neurological lesions. *Am. J. Physiol.*, **203**, 789–795.

SLOAN, J.W., BROOKS, J.W., EISENMAN, A.J. & MARTIN, W.R. (1963). The effect of addiction to and abstinence from morphine on rat tissue catecholamine and serotonin levels. *Psychopharmacologia*, **4**, 261–270.

WAY, E.L. & ADLER, T.K. (1960). The pharmacologic implications of the fate of morphine and its surrogates. *Pharmac. Rev.*, **12**, 383–446.

WAY, E.L., LOH, H.H. & SHEN, F.H. (1969). Simultaneous quantitative assessment of morphine tolerance and physical dependence. *J. Pharmac. exp. Ther.*, **167**, 1–8.

(*Received June 30, 1978.*
Revised October 9, 1978.)