SOME INTERACTIONS BETWEEN MORPHINE AND DIAZEPAM IN THE MOUSE AND RABBIT

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SUMMARY

Some interactions between diazepam and morphine have been examined in the conscious animal, looking particularly at respiratory effects in the rabbit and the relationship between respiratory depression and analgesia in the mouse. Some evidence of potentiation of the respiratory effects of morphine was found in both species. This was manifest as Pco₂ changes in the rabbit and depression of respiratory rate in the mouse. No separation between potentiation of morphine analgesia and respiratory rate depression, by diazepam, was found in the mouse.

Diazepam has been shown to potentiate the effects of several drugs which act on the central nervous system. Respiratory depression by barbiturates is intensified by diazepam both in animals (Southgate and Wilson, 1971) and in man (Prensky et al., 1967; Bell, 1969). Vajda, Prineas and Lovell (1971) have shown a higher incidence of phenytoin toxicity in patients who were given diazepam together with phenytoin and concluded that diazepam caused increased blood levels of phenytoin. Bell (1969) suggested that the synergistic actions of diazepam might be shown to exist for a variety of drugs.

Diazepam is used for premedication in general anaesthesia (Knight and Burgess, 1968) and as an intravenous hypnotic in patients undergoing cardioversion (Glassman, 1971). Thus possible interactions with narcotic analgesics are of particular interest. Hunter (1968) and Hellewell (1968) have noted severe respiratory depression or apnoea in some patients who were given both diazepam and narcotic analgesics. On the other hand, Sadove, Balagot and McGrath (1965) found no change in the respiratory response to pethidine in patients treated with diazepam. Cohen, Finn and Steen (1969) reported similar findings.

In the clinical situation, observations of interactions between narcotic analgesics and diazepam are often complicated by the presence of other drugs.

Methods

Mice.

Groups of male and female mice (25–35 g) of the Alderley Park strain were studied. Analgesia was estimated using the hot plate reaction time test. Each mouse was placed in an open ended perspex cylinder on a hot plate maintained at a temperature of 55°C. The end point was taken as a distinct shake of the hind paw or a jump from the surface of the plate (Beecher, 1957). The reaction time was measured from the initial contact with the plate to the end point. To avoid the possibility of tissue damage any mouse not responding in 45 sec was removed from the plate.

Reaction times were measured at 15-min intervals after the injection of the drug and the tests were continued until the reaction times were not significantly different from those of concurrently tested control mice injected with saline.

Respiratory frequency was measured just before each measurement of reaction time by placing the mouse's snout in the barrel of a 2-ml syringe connected to a pressure transducer and pen recorder. Respiratory movements were recorded for at least 10 sec.

Both respiratory frequencies and reaction times are quoted as mean absolute values (±SE).

Rabbits.

Groups of Dutch rabbits of either sex (weight-range 1.5–2.0 kg) were studied.

Respiratory frequency and minute volume were...
measured using a Krogh spirometer. In general the results have been expressed as percentage change from the control measurement, but some absolute values have been included to allow comparison with results from other workers.

Blood PCO₂, pH and standard bicarbonate were measured using the Astrup technique. The blood was obtained from the marginal vein of a warmed ear. The applicability of these measurements to the rabbit has been described by Rees (1967).

At least two consistent control readings were obtained before injection of the drug and measurements were repeated at 15, 30 and 60 min after injection and at hourly intervals until they had returned to the control values. The rectal temperature was measured at hourly intervals using a rectal thermometer so that corrections could be applied to the Astrup measurements if necessary.

The results were expressed as mean change from control values (±SE). The significance of differences between the means was estimated using the Student t-test.

Drugs used.

Diazepam was used as a solution in the commercially available solvent (Valium, Roche Products Ltd).

Diazepam solvent was prepared from a formula supplied by Roche Products Ltd and contained: propylene glycol (45% v/v), ethanol (10% v/v), benzyl alcohol (1.5% v/v), sodium benzoate (9.8% w/v), benzoic acid (0.24% w/v) in water.

Morphine sulphate was used as an aqueous solution supplied by Evans Medical Ltd (20 mg/ml). Dilutions, where necessary, were made with saline (0.9%).

In mice all injections were administered by the intraperitoneal route. In rabbits all injections were administered intravenously.

RESULTS

Mice.

The effects of morphine, diazepam and diazepam solvent alone and in combination upon the maximum hot plate reaction time and the minimum respiratory frequency exhibited by the mice are shown in table I. Neither diazepam alone, up to 2.5 mg/kg, nor diazepam solvent had any effect on these measurements.

Morphine, 10 and 20 mg/kg, significantly reduced respiratory frequency and increased hot plate reaction time (P<0.001). These effects were significantly increased (P<0.05) by concurrent administration of 1.25 or 2.5 mg/kg diazepam. This increase was not seen with the diazepam solvent alone.

Figure 1 shows the time course of the effects of diazepam 2.5 mg/kg and morphine 20 mg/kg alone and in combination on the respiratory frequency and hot plate reaction time. The combination of diazepam with morphine prolonged both the respiratory depression and analgesic action of morphine. Throughout the time course of action of the two drugs increases in analgesic activity were always associated with increases in respiratory depression. Thus no separation between analgesia and depression of respiratory frequency occurred when morphine was given in combination with diazepam in the mouse.

A similar pattern was seen with other dose combinations of morphine and diazepam except that the prolongation of morphine's action was less marked with diazepam 1.25 mg/kg.

At no time, in the time course of action of these drugs, did mice pretreated with diazepam alone produce results significantly different from saline controls.

Rabbits.

Respiratory minute volume (V) and respiratory frequency (f). Control resting V was 470 ± 50 ml/min and control f was 114 ± 14 b.p.m. in a group of 8 rabbits. Over a 5-hour period the injection of 0.5 ml/kg saline had no effect upon V or f.

The effects of morphine 4 mg/kg, diazepam 4 mg/kg and a combination of these two drugs on V and f are shown in figure 2. Rabbits treated with
the combination of drugs showed a more prolonged period of depression of \( V \) and \( f \) than did rabbits treated with morphine alone. The maximum depression of minute volume was enhanced. However, rabbits injected with diazepam 4 mg/kg alone exhibited respiratory depression for the total duration of the experimental period.

Lower doses of diazepam (1 and 2 mg/kg) in combination with morphine gave values which lay between those for morphine alone and in combination with 4 mg/kg diazepam (see fig. 2).

\( P_{CO_2} \), \( pH \) and standard bicarbonate changes. The mean control values for a group of 8 rabbits were:

\[ P_{CO_2} = 34.0 \pm 0.8 \text{ mm Hg}; \ \ \text{pH} = 7.443 \pm 0.010; \ \ \text{standard bicarbonate} = 24.4 \pm 0.4 \text{ m.equiv/l}. \]

Saline injected control rabbits showed a slight increase in \( \text{pH} \) and a fall in \( P_{CO_2} \) and standard bicarbonate during the 5-hour experimental period. None of the changes was statistically significant.

The time course of changes in the \( P_{CO_2} \) and \( \text{pH} \) of blood from rabbits treated with diazepam 4 mg/kg, morphine 4 mg/kg or a combination of these two drugs is shown in figure 3. Changes in standard bicarbonate are shown in table II.

Diazepam 4 mg/kg alone caused a small rise in
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Pco₂. This was significantly greater than the Pco₂ of saline-injected control rabbits 30 min after injection (P<0.05). The increase in pH was significantly greater than that of saline-injected rabbits 1 hour after injection (P<0.01) and was associated with an increase in standard bicarbonate (P<0.001).

Morphine 4 mg/kg alone caused an increase in Pco₂ and a fall in blood pH lasting 3 to 4 hours. No statistically significant changes in standard bicarbonate were observed.

In combination with diazepam, morphine produced a significantly greater and more prolonged increase in Pco₂. There was no significant change in the effects of morphine on blood pH. Thirty min after injection standard bicarbonate values for rabbits injected with morphine and diazepam were significantly lower (P<0.05) than those of rabbits treated with morphine alone. One to 4 hours after injection, standard bicarbonate values for rabbits injected with the two drugs were increased. There were no significant changes in the rectal temperature of rabbits injected with diazepam and morphine over a 5-hour period.

**DISCUSSION**

The activity of morphine as an analgesic, as measured by the hot plate test, and as a depressant of respiratory frequency in the mouse was increased when combined with diazepam. The doses of diazepam used had no effect on either hot plate reaction time or respiratory frequency and thus diazepam potentiates rather than summates with the actions of morphine in the mouse. There was no separation between analgesia and respiratory depression and so any advantage of potentiation of analgesia was counteracted by concurrent potentiation of respiratory depression. In man, evidence for the potentiation of the analgesic action of narcotic analgesics by diazepam is sparse. Flowers, Rudulph and Desmond (1969) have reported lower requirements for pethidine in patients receiving diazepam in labour. However, this is unlikely to be potentiation of pethidine by diazepam as diazepam alone appeared to reduce the need for an analgesic.

In the rabbit diazepam alone caused a fall in V and f confirming findings reported previously by two of us (Bradshaw and Pleuvry, 1971). The greatest effect was on V rather than f. Similar changes in respiration after diazepam have been reported in man. Using diazepam as an intravenous induction agent, McClish (1966) reported a slight degree of

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**FIG. 3.** Time course of the effects of morphine and diazepam on the pH and Pco₂ of the blood obtained from the marginal ear vein of the rabbit. The results are expressed as mean change ± SE of groups of 5-8 rabbits. D = diazepam 4 mg/kg; M = morphine 4 mg/kg; D + M = diazepam 4 mg/kg and morphine 4 mg/kg in combination.

**TABLE II.** Effect of diazepam and morphine, alone and in combination, on the standard bicarbonate in the rabbit. Results are expressed as means ± SE of groups of 5-8 rabbits.

<table>
<thead>
<tr>
<th>Time after injection</th>
<th>Diazepam 4 mg/kg</th>
<th>Morphine 4 mg/kg</th>
<th>Morphine + Diazepam</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>+0.7 ±0.6</td>
<td>-0.1 ±0.6</td>
<td>-1.2 ±0.5</td>
</tr>
<tr>
<td>30 min</td>
<td>+0.9 ±0.8</td>
<td>+0.2 ±0.4</td>
<td>-1.4 ±0.5</td>
</tr>
<tr>
<td>1 hour</td>
<td>+3.1 ±0.8</td>
<td>-0.2 ±0.6</td>
<td>+0.4 ±1.1</td>
</tr>
<tr>
<td>2 hours</td>
<td>+1.7 ±1.0</td>
<td>+0.3 ±1.2</td>
<td>+3.1 ±1.1</td>
</tr>
<tr>
<td>3 hours</td>
<td>+0.6 ±0.4</td>
<td>-0.2 ±0.6</td>
<td>+1.6 ±1.4</td>
</tr>
<tr>
<td>4 hours</td>
<td>+0.3 ±0.5</td>
<td>-0.6 ±0.8</td>
<td>+2.5 ±1.3</td>
</tr>
<tr>
<td>5 hours</td>
<td>-0.2 ±1.3</td>
<td></td>
<td>-0.1 ±1.2</td>
</tr>
</tbody>
</table>
respiratory depression, this being a reduction of tidal volume rather than frequency. Hunter (1967) made a similar observation. Parkes (1968) ascribed the effects of diazepam on the respiration of the rabbit to a calmer state of the animal rather than respiratory depression as typified by morphine. This view is supported by the very small (3.5 mm Hg) and transient increase in Pco₂ exhibited by rabbits treated with diazepam alone.

Diazepam did not affect the depression of respiratory frequency induced by morphine and the effect on minute volume could be interpreted as summation. However, it would be difficult to ascribe the effect of the combination of morphine and diazepam on Pco₂ to a summation of effects. Four hours after injection, a time when diazepam alone has no significant effect upon Pco₂, rabbits injected with both morphine and diazepam had Pco₂ values more than 10 mm Hg above those of rabbits injected with morphine alone.

It is clear that there are species variations in the interaction of morphine and diazepam. In mice the effects of morphine upon respiratory frequency were potentiated, but in rabbits respiratory frequency depression was unaffected. However, in at least two species, rabbit and mouse, the combination of morphine and diazepam have effects upon respiratory parameters which are difficult to explain as simple summation. It is possible that a similar situation applies to other species including man. Perhaps morphine should be included with the barbiturates, phenytoin and warfarin as a drug with which diazepam may be synergistic.

Some workers have investigated the possibility of enzyme inhibition playing a part in the potentiation of the actions of other drugs by diazepam. The increased blood levels of phenytoin in patients receiving diazepam as well as phenytoin (Vajda, Prinças and Lovell, 1971) suggests this possibility. However, Jori, Prestini and Pugliatti (1969) investigated the effects of diazepam on a variety of drug-metabolizing enzymes in rat liver. Even in high doses diazepam did not inhibit the metabolic degradation of p-nitroanisol, aniline, aminopyrine or pentobarbitone. Indeed repeat administration of diazepam produced some evidence of enzyme induction.

Nevertheless both diazepam (Randall, Scheckel and Pool, 1970) and morphine (Goodman and Gilman, 1970) are n-demethylated in vivo and thus it is possible that these two drugs compete for the same enzyme system causing mutual inhibition of metabolism.

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REFERENCES

SOCIETY FOR THE ADVANCEMENT OF MEDICAL ENGINEERING

The Society for the Advancement of Medical Engineering (SAME) is to organize a series of training courses for hospital technicians.

The aim of the courses is to provide bio-engineering training for paramedical staff with particular emphasis on the maintenance and operation of advanced technology equipment.

The first course will be devoted to ventilators and will be held at Himley Hall, near Birmingham from February 20 to 22, in conjunction with the Birmingham Regional Hospital Board. It will be open to technicians of HNC or equivalent standard.

Topics to be covered include the physiological principles of ventilation, standards and methods of testing ventilators, and main principles of maintenance.

Speakers include Professor J. S. Robinson of Queen Elizabeth Hospital, Birmingham, Professor M. K. Sykes of Hammersmith Hospital, and Dr J. A. Bushman of the Royal College of Surgeons.

There will be 30 places available and the cost will be £35 per person. Full details and application forms are available from Miss R. Linzell, Secretary, SAME, Hammersmith House, London W6 9DX (telephone 01-748 2020).