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The effects of nefopam on blood acid-base status in the rabbit: interactions with morphine in the mouse and rabbit

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Nefopam, a non-narcotic analgesic, had only minor effects upon the blood pH, P_{CO_2} and standard bicarbonate of the conscious rabbit. In contrast morphine caused a dose-dependent increase in P_{CO_2} and standard bicarbonate. When nefopam was administered in conjunction with morphine (2·5 mg kg^{-1} and 1 mg kg^{-1} i.v. respectively) the increase in P_{CO_2} was greater than with morphine alone. Nefopam also increased the antinociceptive and respiratory frequency depressant effects of morphine in the mouse.

Nefopam is a cyclized analogue of orphenadrine whose original pharmacological profile suggested antidepressant (Bassett et al 1969) and central muscle relaxant (Klohs et al 1972) activity. Clinical trials revealed, however, that nefopam had significant analgesic activity by both parenteral and oral routes (Cohen 1974; Gassel et al 1976). Nefopam does not appear to be either structurally or pharmacologically related to any other known analgesic groups. Since the analgesic activity of nefopam has been compared with the narcotic analgesics (Sunshine & Laska 1975; Tigerstedt et al 1977) attention has been drawn to possible respiratory effects of nefopam. Gasser & Bellville (1975) found that nefopam caused little depression of the ventilatory response to CO_2 when examined as mean effects in six volunteers. However, there was considerable variation in response within the group, and one volunteer who received higher than the recommended dose exhibited marked displacement of the CO_2 response line.

The present study was designed to examine the full dose response relationship for nefopam on blood gas status in the rabbit. The effects of nefopam were to be compared with those of morphine, an analgesic with well documented respiratory depressant activity, and interactions between the two drugs investigated.

Since the therapeutic use of nefopam is as an analgesic, interactions with morphine have also been examined using analgesic tests in mice.

METHODS

Male and female, half lop white rabbits (2·5 to 4 kg) were used to measure pH and P_{CO_2} of arterialized venous blood. It is possible to obtain arterial samples from a rabbit by the use of cannuli inserted into either the carotid or femoral artery. This procedure requires prior surgery to the rabbit and it was felt that this was unjustified in view of our preliminary findings that there was no significant difference between the P_{CO_2} and pH of blood obtained from the femoral artery and that obtained from the lateral ear vein treated as described below (Rees 1966). The rabbits were gently restrained by wrapping them in a towel with only the head protruding. The ears were shaved and kept warm throughout the experiment by proximity to an electric light. Blood samples were taken from the lateral ear vein, which was warmed and massaged to ensure brisk circulation. Blood was collected anaerobically into heparinized capillary tubes, thoroughly mixed and drawn into a Radiometer blood micro system (BMS 3, Mk 2). The Radiometer blood microsystem was calibrated using a Gas mixing apparatus GMA 1 which delivers 3·59 ± 0·04% and 7·21 ± 0·07% CO_2. The pH electrode was calibrated using BDH Buffer solutions for blood pH analysis 7·380 ± 0·005 and 6·840 ± 0·005 at 38 °C. When calibrated in this way the apparatus has an accuracy of ±0·001 for pH and ±0·013 kPa for P_{CO_2}. P_{CO_2} and pH were measured directly and standard bicarbonate was calculated using a Siggaard-Andersen alignment nomogram assuming a normal haemoglobin concentration of 12·5 g $litre^{-1}$. Whilst this assumption means that the absolute values of standard bicarbonate might be inaccurate, it is possible to detect changes which occur during the experimental period.

Before drugs were administered, repeat control measurements of blood gas were made until con-

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sistent values were obtained. Drugs were administered intravenously slowly over 1 min. After drug administration blood samples were collected at 5 and 15 min after injection and then at 15 min intervals until three consecutive samples showed no further change in blood P_{CO_2} and pH.

Antinociceptive activity was measured using groups of twelve female albino mice (25–35 g, Manchester strain). The hot plate reaction time test was used as described by Bousfield & Rees (1969) and any mouse not reacting in 45 s was removed from the hot plate to prevent tissue damage. Reaction times were measured at 15 min intervals after the intraperitoneal injection of drug. Experiments were continued until drug-treated mice had reaction times not significantly different from concurrently tested, 0·9% NaCl (saline)-treated controls.

Respiratory frequency was measured by placing the mouse's snout into the barrel of a 5 ml syringe connected to a pressure transducer and pen recorder. Respiration was monitored for not less than 10 s. Respiratory frequency was measured before injection of the drug and subsequently before each measurement of reaction time.

The drugs used were morphine hydrochloride and nefopam hydrochloride. Solutions were prepared by dissolving the powders in sterile water for injection.

Unless otherwise stated, results are expressed as means and standard errors of either 6 (rabbits) or 12 (mice) experiments. Significance was assessed using the Student's *t*-test or the Mann Whitney U test.

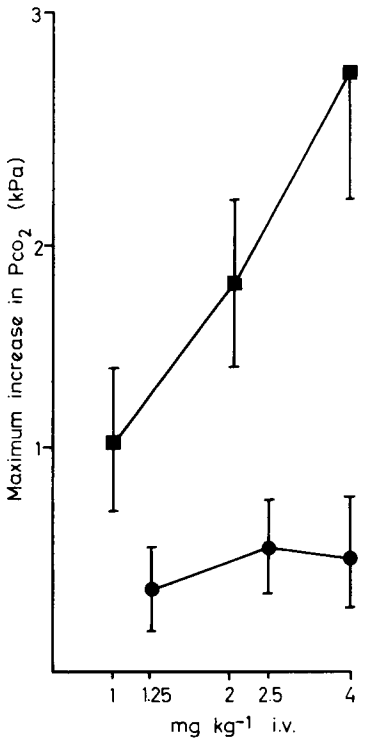

FIG. 1. Dose response relationships for morphine (closed squares) and nefopam (closed circles) on the arterialized venous blood P_{CO_2} of the conscious rabbit. The results are expressed as the maximum mean effect ± standard error of not less than 6 experiments.

RESULTS

Arterialized venous pH and P_{CO_2}

Pre-drug control values, for example in a group of 6 rabbits to be given 1 mg kg^{-1} morphine, were: pH 7·478 ± 0·016; P_{CO_2} 4·92 ± 0·11 kPa; standard bicarbonate 26·0 ± 0·11 mmol l litre^{-1}.

Nefopam in doses of 5 mg kg^{-1} and above caused convulsions in all rabbits. This was associated with a fall in P_{CO_2} (−0·61 ± 0·11 kPa) pH (−0·336 ± 0·054) and standard bicarbonate (−15·4 ± 1·12 mmol) litre^{-1} (n = 3). No further experiments were carried out at this dose level.

1·25 mg kg^{-1} nefopam had no significant effects on the pH, P_{CO_2} and standard bicarbonate of the rabbit, when compared with rabbits treated with saline. However, 2·5 and 4 mg kg^{-1} nefopam caused significant rises in P_{CO_2} during the first hour after injection, but this was considerably less than the changes which occurred after morphine treatment (Fig. 1). Doses of nefopam below 5 mg kg^{-1} caused no significant changes in pH and only the highest dose of morphine, 4 mg kg^{-1}, caused a significant fall during the first hour after injection. For example 15 min after 4 mg kg^{-1} morphine, arterialized venous blood pH had fallen by 0·103 ± 0·030 compared with saline values of −0·016 ± 0·008. The relative lack of change in pH of morphine treated rabbits exhibiting marked changes in P_{CO_2} was due to concurrent increases in standard bicarbonate values (Fig. 2). In contrast, nefopam caused a transient fall in standard bicarbonate. For example, 5 min after 2·5 mg kg^{-1} nefopam standard bicarbonate fell 3·5 ± 0·8 mmol l^{-1} compared with saline-induced changes of + 0·6 ± 1·2 mmol litre^{-1}.

The combination of 1 mg kg^{-1} morphine and 2·5 mg kg^{-1} nefopam caused a greater increase in P_{CO_2} than morphine alone (Fig. 3a). The increase in bicarbonate due to morphine was unaffected by the addition of nefopam (Fig. 3b) and thus the fall in pH produced by the combination was greater than that seen with each drug individually (Fig. 3c).

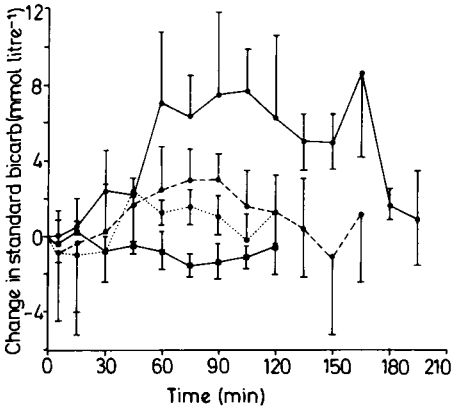

FIG. 2. The effects of morphine (closed circles) on the standard bicarbonate of arterialized venous blood from the conscious rabbit. Dose are 1 mg kg^{-1} (dotted line), 2 mg kg^{-1} (dashed line) and 4 mg kg^{-1} (solid line). Changes occurring in saline control rabbits (closed squares) are shown for comparison. Results are means ± standard errors of 6 experiments.

Antinociceptive activity

Via the intraperitoneal route nefopam caused convulsions in mice with doses of 20 mg kg^{-1} and over. Below these doses it caused a dose-dependent increase in reaction time. However the maximum effect of nefopam in this test was an increase in reaction time of 12·83 ± 3·4 s 45 min after 10 mg kg^{-1} nefopam. Morphine 20 mg kg^{-1} caused most mice to remain on the hot plate until the arbitrary cut off of 45 s.

At no dose level did nefopam significantly depress respiratory rate, indeed there was a tendency for the frequency of respiration to be increased (Fig. 4).

In all the dose combinations tested, nefopam increased both the analgesic and respiratory frequency depressant effects of morphine. Fig. 4 is an example of these results.

DISCUSSION

Even in doses close to the convulsant dose, nefopam has minimal effects on acid base status in the rabbit. The maximum rise in arterialized venous P_{CO_2} was 0·5 kPa. Similar changes have been reported during physiological sleep (Ostergaard 1944). In contrast, morphine caused a dose-dependent increase in arterialized venous P_{CO_2} and standard bicarbonate. These effects of morphine in the rabbit have been reported previously by Rees (1967).

In mice, nefopam had no significant effects upon respiratory rate in subconvulsant doses, whilst morphine produced a marked fall in respiratory

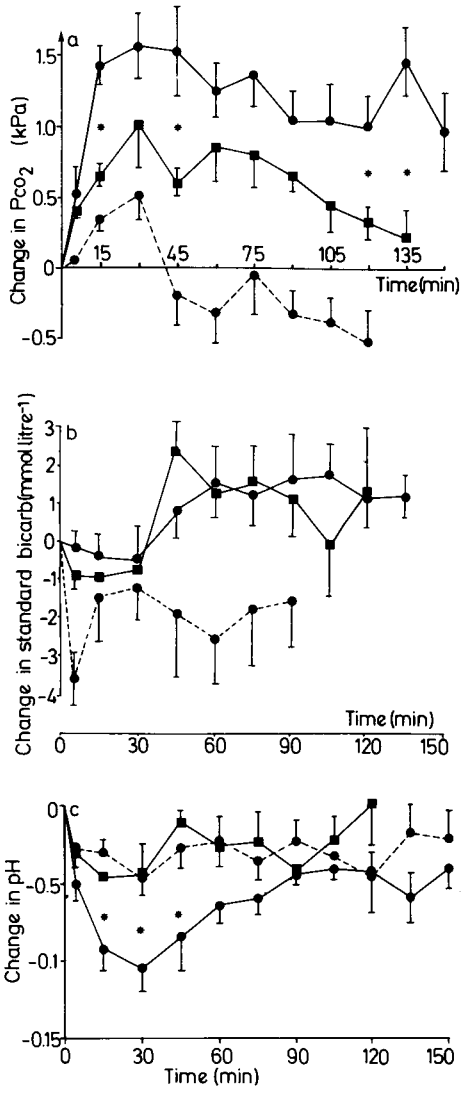

FIG. 3. The effect of morphine 1 mg kg^{-1} (closed squares) and nefopam 2·5 mg kg^{-1} (closed circles, dashed line) alone and in combination (closed circles, solid line) on the (a). P_{CO_2} (b). standard bicarbonate and (c). pH of arterialized venous blood from the conscious rabbit. Results are expressed as means ± standard errors of 6 experiments.

* Significantly different from morphine alone $P < 0·05$.

frequency. Thus in both mice and rabbits nefopam appears to have little respiratory depressant activity.

Unfortunately nefopam also possessed less peak analgesic activity than morphine using the mouse hot plate reaction time test. Whilst it has been reported that nefopam has variable analgesic activity in some animal tests (Hammerbeck et al 1974), recent clinical

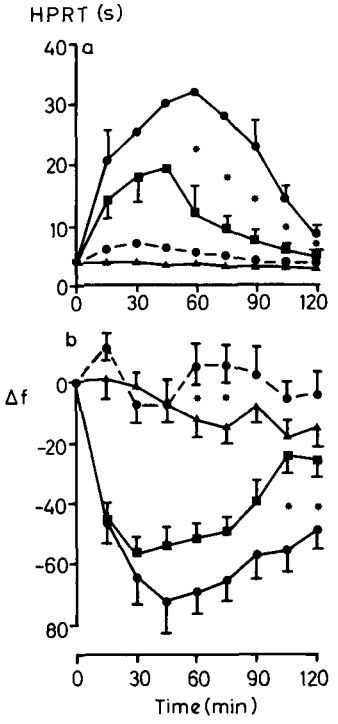

FIG. 4. The effect of morphine 5 mg kg^{-1} (closed squares) and nefopam 5 mg kg^{-1} (closed circles, dashed line) alone and in combination (closed circle, solid line) on (a) hot plate reaction time and (b) respiratory frequency in the mouse. Saline control responses (solid triangles) are shown for comparison. Results are expressed as mean values ± s.e. of 12 experiments; s.e.s are not shown on reaction times containing values exceeding 45 s or on the values for saline and nefopam alone which did not exceed the confines of the symbol.
* Significant difference between values $P < 0.05$.

findings have also suggested that nefopam has a low peak analgesic activity (Tigerstedt et al 1979), when compared with another opioid, oxycodone. The main use for nefopam would seem to be for mild to moderate pain.

The possibility that nefopam might enhance morphine-induced analgesia without enhancing the respiratory depression was also examined in animals. Nefopam did significantly enhance morphine's analgesic action measured as hot plate reaction time in mice, but it also enhanced morphine's respiratory effects measured as depression of respiratory frequency in mice and increase in P_{CO_2} in rabbits.

The combined effects of the two drugs on arterialized venous P_{CO_2} was not simply addition of their individual actions since the P_{CO_2} was still elevated two to three hours after the combined injection. At this time both morphine and nefopam alone had no significant effects upon P_{CO_2} (Fig. 3).

It is possible that nefopam affects the metabolism of morphine thus enhancing its action. However, nefopam did not enhance the actions of morphine on standard bicarbonate and the fall in pH seen after the two drugs was equivalent to that seen with four times the dose of morphine when given alone. It is unlikely that reduced metabolism of morphine could explain these changes.

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