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The effects of etorphine and of morphine on respiration, blood carbon dioxide tension, and carbon dioxide sensitivity in the conscious rabbit

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The effects of intravenous doses of morphine and etorphine on respiration, blood pCO_2 and sensitivity to carbon dioxide were investigated in the conscious rabbit. Both morphine and etorphine depressed respiratory rate and elevated pCO_2. Etorphine was about 5000 times more potent than morphine as a depressant of respiratory rate. With etorphine, depression of respiratory rate was always accompanied by a large increase in tidal volume. In the normal rabbit, inhalation of mixtures of carbon dioxide up to 15% (in oxygen) produced a concentration-dependent increase in tidal volume accompanied by no increase in respiratory rate. After injection of morphine followed by inhalation of carbon dioxide there was a dose-dependent depression of carbon dioxide sensitivity which was concurrent with the depression of respiratory rate; 8 mg/kg completely abolished the respiratory stimulant effects of concentrations of carbon dioxide up to 12%. Whilst high doses of etorphine depressed carbon dioxide sensitivity, a dose of 0·5 μg/kg (which depressed respiratory rate by about 30%) did not depress carbon dioxide sensitivity. Doses of 1 μg/kg of etorphine administered to pregnant animals produced an increase in respiratory rate and a depression of tidal volume. The significance of the differences between the respiratory pharmacology of etorphine and that of morphine is discussed.

The effects of several narcotic analgesics on pH homoeostasis in the rabbit have been described (Rees, 1967; 1968). The present report describes the respiratory pharmacology of the highly potent narcotic analgesic etorphine in the conscious rabbit in which blood carbon dioxide tension (pCO_2), pH, standard bicarbonate, respiratory minute volume and respiratory rate were measured concurrently. The effects of inhalation of carbon dioxide (CO_2) on the respiratory depression produced by etorphine (M99) is also described and compared to those observed after morphine.

EXPERIMENTAL

Apparatus

pH studies. The apparatus was identical to that used by Rees (1967), based upon the method of Astrup, Jørgensen & others (1960).

Respiratory measurements. The method of Gaddum (1941) was used to determine respiratory rate and minute volume. This method measures pressure changes across a capillary which is acting as a resistance to inspiratory flow. Measurements were

made while applying an air-tight face mask over the snout of the animal intermittently for periods of between 1 and 2 min, a procedure which the animals tolerated better than continuous application. Application of the face mask did not alter respiratory rate. Pressure changes were recorded via a tambour on a smoked drum. The apparatus was calibrated against a water manometer using a suction pump which was periodically calibrated against a spirometer.

Method

Groups of not less than four and not more than twenty Flemish rabbits, weighing 2·5–3·5 kg were used. Each group consisted of an equal number of male and female animals. In nearly every instance in which the effects of etorphine were investigated on pH homoeostasis each rabbit was used on only one occasion. No rabbit was used more than twice.

Controls. Before the determination of control values each rabbit was allowed 20 min for its respiration to settle. Although the rabbits were unrestrained they were encouraged to remain stationary, and those for whom such encouragement was inadequate were not used. During the following 20 min two or three control values for blood pCO_2, pH and standard bicarbonate were determined by the method of Astrup & others (1960) in the blood obtained from the marginal vein of the warmed ear (Rees, 1967). Immediately after each blood sampling the control values for respiratory rate, minute volume and tidal volume were obtained.

Effect of changing the diameter of the capillary resistance in the Gaddum apparatus. A capillary bore of 2·00 mm was used routinely, but in some experiments capillary bores of 1·75 and 2·25 were also used. Each capillary was calibrated and then used in succession to determine respiratory minute volume.

Effects of inhaled gases. The exact concentration of CO_2 in mixtures containing approximately 5, 8 and 15% CO_2 (in O_2) was measured either by Haldane's method or by the use of an infrared CO_2 analyser. Mixtures of these CO_2/O_2 concentrations were fed into the Gaddum respirometer circuit avoiding positive pressure. Respiratory rate and minute volume were recorded until no further increase in minute volume was seen over a 30 s period (between 2 and 3 min exposure) both in control animals and in animals treated with morphine or etorphine. The effects of inhalation of 100% O_2 on the respiratory effects of morphine and etorphine were also investigated.

Time course of investigations. After stable control values for all parameters had been obtained drugs were administered intravenously. Samples of blood were obtained 7 and 15 min after injection and then at 15 min intervals until control values were regained. Respiratory rate, minute volume and the effects of inhaled gases were measured immediately after blood sampling. In some experiments changes in respiratory rate and minute volume were recorded continuously for the first 15 min after injection. The effect of repeated doses of etorphine was also investigated in a group of animals. Etorphine, 0·5 μg/kg, was injected and minute volume and rate followed until a peak change in rate was recorded at which time a second dose of 0·5 μg/kg was administered and the same procedure followed until a maximum change was observed. A dose of 1·0 μg/kg was then injected and when peak change in rate was recorded blood was sampled and pCO_2 measured. In all instances the interval between injections was between 5 and 10 min.

Expression of results. Changes in respiratory rate and minute volume are expressed as percentage change from control values. Changes in tidal volume were calculated

from these two parameters and expressed in the same way. Changes in pH, pCO_2 and standard bicarbonate are expressed as the difference between each respective reading and its control.

Drugs

Drugs used were morphine sulphate injection B.P. (BDH) and etorphine hydrochloride [7α(1-(*R*)-hydroxy-1-methylbutyl)-6,14-*endo*ethenotetrahydro-oripavine hydrochloride] (Reckitt & Sons Ltd.). A stock solution of 10 μg/ml was buffered at pH 4·0. Dilutions of both drugs were made in sterile saline. All doses are expressed in terms of the salts.

RESULTS

Effect of changing the diameter of the capillary resistance. Fig. 1 shows the calibration slopes of mm excursion of the pointer against air flow in ml/min for each of the three capillary resistances. The relation was linear up to about 1200 ml/min. Respiratory minute volumes in excess of this figure were rarely encountered in these experiments. Table 1 shows the changes in respiratory minute volumes measured with each of the capillaries in a variety of experimental types (some of which are not described in this report). There was no significant difference between changes in respiratory minute volumes calculated from each capillary nor was there a consistent trend in the values related to the capillary bore.

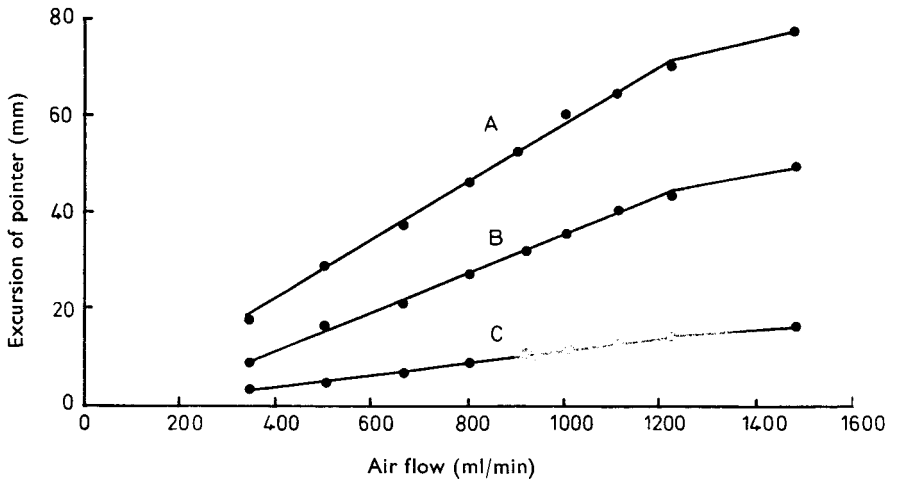

FIG. 1. The relation between airflow through the Gaddum apparatus and the excursion of the pointer, measured using three capillary tubes of different bore. A = a capillary of internal diameter 1·75 mm; B = internal diameter 2·00 mm; C = internal diameter 2·25 mm.

Effects of inhaled CO_2 in control animals. The percentage changes in respiratory minute volume, rate and tidal volume on exposure of rabbits to various concentrations of CO_2 up to 15% are shown in Fig. 2. The inhalation of 5 or 8% CO_2 had little effect on respiratory rate, but 15% produced a fall. On the other hand, increasing the ambient CO_2 percentage produced an increase in tidal volume roughly proportional to the concentration of CO_2. At 15% this represented a 2·5-fold increase. The net effect on minute volume was therefore a concentration-dependent increase.

Table 1. *The values of percentage change in minute volume recorded under a variety of experimental conditions during which three different capillary resistances were incorporated into the Gaddum apparatus and used consecutively. Means of 4 animals ± s.e.*

Experiment	Resistance (letters refer to key in Fig. 1; internal capillary diameter is given in mm) A (1·75 mm)	B (2·00 mm)	C (2·25 mm)
% depression of respiratory minute volume produced by 4 mg/kg morphine	31·0 ± 1·1	30·9 ± 1·8	29·7 ± 2·3
% depression of minute volume produced by 30 mg/kg pentobarbitone (*)	37·9 ± 2·2	39·1 ± 1·7	39·5 ± 2·7
% increase in respiratory minute volume response to 12% CO_2 after 4 mg/kg morphine	34·4 ± 1·8	38·0 ± 2·6	36·8 ± 2·2
% increase in respiratory minute volume response to 12% CO_2	51·1 ± 1·4	53·9 ± 1·0	54·9 ± 1·7
% increase in respiratory minute volume response to 7·2% CO_2 in animals pretreated with progesterone (*)	42·7 ± 1·0	41·4 ± 1·4	41·4 ± 1·1
% increase in respiratory minute volume response to 4% CO_2 in animals pretreated with progesterone (*)	30·5 ± 1·2	29·7 ± 1·8	29·7 ± 1·2

(*) Readings taken from studies not described in this paper.

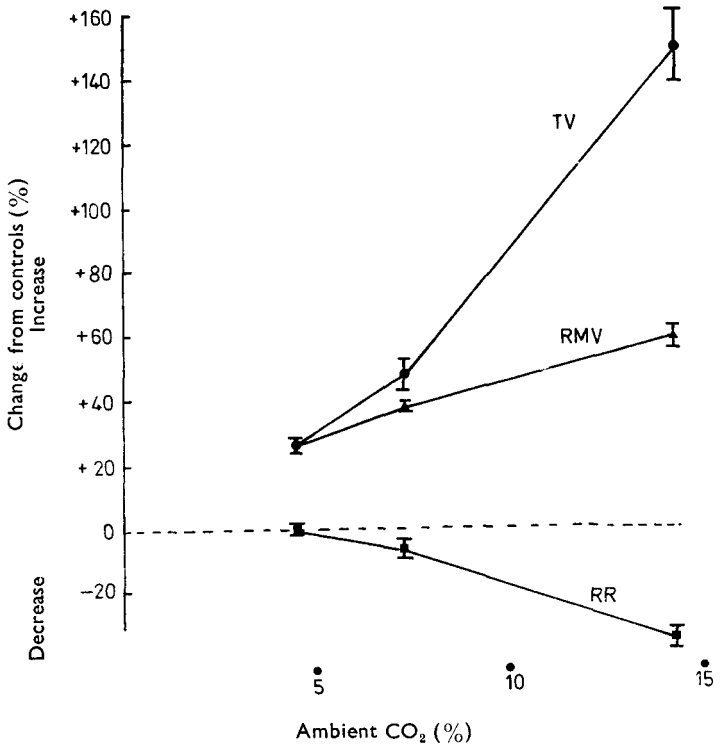

FIG. 2. The effect of elevating the ambient carbon dioxide concentration on tidal volume (circles, TV), respiratory minute volume (triangles, RMV) and respiratory rate (squares, RR) in the conscious rabbit. Means of at least 20 determinations ± standard error.

Effects of etorphine—gross observations. The most obvious effect observed with the doses used in this series (0·25–8·0 μg/kg) was sedation, but no doses produced unconsciousness. With doses of 2 μg/kg and above, a characteristic catatonic state developed a few minutes after injection, and persisted for about a quarter of an hour. With doses of 4 μg/kg some rabbits convulsed two or three times during the first 5 min after injection. These convulsions were less severe than those seen after pethidine in high doses in the rabbit. At a dose of 8 μg/kg three of the four animals convulsed. The fourth showed a catatonic condition and this was the only animal which died.

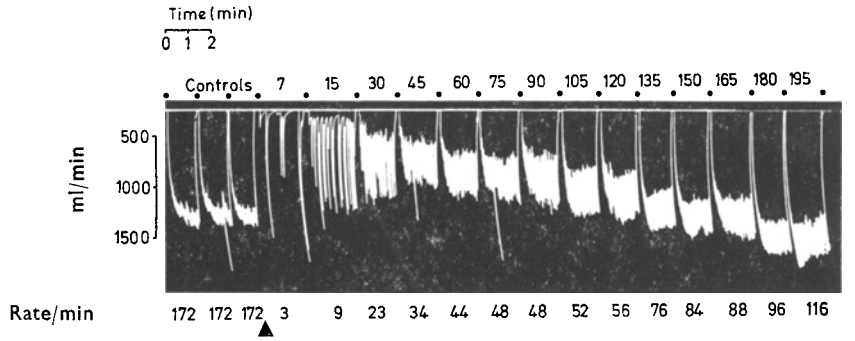

FIG. 3. The effects of 2 μg/kg etorphine on respiratory rate and minute volume measured by the Gaddum respirometer. Minute volume calibration is shown on the left, and respiratory rate is recorded below the trace. Recordings were made during the application of a face mask for periods of between 1 and 2 min (see time scale). At the black circles the face mask was removed and the drum stopped. The time after injection at which recordings were made is stated above the trace. At arrow, etorphine, 2 μg/kg.

Effects of etorphine on respiration. Fig. 3 shows a characteristic recording of the effects of 2 μg/kg on respiratory minute volume and rate. Three control readings are shown. Seven min after injection, the irregular and infrequent gasping respiration

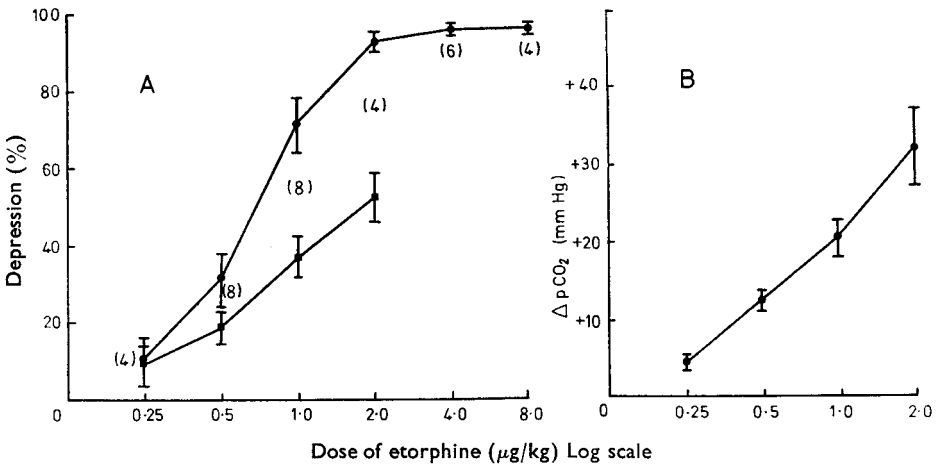

FIG. 4 A. The relation between the dose of etorphine and the percentage depression of respiratory rate (circles) and minute volume (squares) in groups of rabbits. The numbers in brackets refer to the number of rabbits in each group. Means are ± standard error.

B. The relation between the dose of etorphine and the maximal elevation of blood pCO_2 Means are ± standard error.

can be seen during which no accurate determination of minute volume could be made. A marked increase in tidal volume is much in evidence, for although minute volume had returned to the control value 150 min after injection, respiratory rate at this time was still only 49% of the original control.

Cheyne Stokes respiration was frequently observed at all doses. At the highest dose rabbits respired 5 to 10 times during a period of about 10 s, this being followed by an apnoeic period of between 40 and 50 s.

Log dose response relations for both respiratory rate and minute volume are shown in Fig. 4A. Respiratory rate only could be determined at doses in excess of 2 μg/kg because the convulsions produced at these doses precluded application of the face mask. The percentage depression of respiratory rate was always greater than that of minute volume. The maximum change in tidal volume for each dose of etorphine is shown in Table 2. There was a dose-dependent increase in tidal volume.

Table 2. *The maximum change in tidal volume after injection of etorphine in the rabbit*

Dose of etorphine (μg/kg)	Increase in tidal volume (% ± s.e.)	Time after injection (min)	Number of experiments
0·25 (mixed group)	6 ± 4	15	4
0·5 (male group)	14 ± 6	15	4
0·5 (female group)	38 ± 10	7	4
1·0 (male group)	190 ± 73	7	4
1·0 (female group)	170 ± 56	7	4
2·0 (mixed group)	344 ± 44	7	3

Changes in minute volume, rate and tidal volume during the first 15 min after injection of 1 μg/kg are shown in Table 3. The increase in tidal volume appeared immediately after the injection.

Table 3. *Changes in respiratory minute volume, respiratory rate and tidal volume during the first* 15 *min after intravenous injection of* 1 μg/kg *of etorphine into a group of four rabbits*

Time after injection (min)	Decrease in respiratory rate (% ± s.e.)	Decrease in respiratory minute volume (% ± s.e.)	Increase in tidal volume (%)
1	60·1	51·3 ± 5·8	83
2	68·7 ± 4·8	50·8 ± 0·9	201
3	73·1 ± 9·0	52·4 ± 1·0	163
4	74·6 ± 10·8	55·8 ± 2·6	256
5	71·6 ± 9·2	47·5 ± 2·8	189
6	67·2 ± 12·5	45·7 ± 5·3	164
7	68·1 ± 13·0	44·4 ± 6·0	178
8	64·5 ± 16·1	40·5 ± 4·3	190
9	66·9 ± 12·7	37·2 ± 4·9	195
10	65·7 ± 13·8	33·9 ± 5·5	200
15	59·4 ± 16·2	27·7 ± 7·7	140

When 2 μg/kg was injected by the cumulative method (0·5 + 0·5 + 1·0 μg/kg) changes in minute volume and rate were not significantly different from those produced by the same dose given as a single injection. The mean maximum depression of minute volume (± s.e.) when the drug was injected as a single dose was 52·1 ± 6·0%

whilst that produced by cumulative injections was 50·5 ± 1·7%. The respective values for depression of respiratory rate were 93·3 ±2·0 (single injection) and 86·3 ± 6·6 (cumulative injections).

Effects of etorphine on pH homoeostasis. Etorphine caused an increase in blood pCO_2 which was linearly related to log dose (Fig. 4B). When change in respiratory minute volume or tidal volume was plotted against the concurrent change in pCO_2, only the change in tidal volume against change in pCO_2 was found to be linear and passing through the origin.

Table 4. *The maximum fall in blood pH and the concurrent* changes in standard bicarbonate and pCO_2 after intravenous injection of etorphine into groups of four rabbits*

Dose of etorphine (μg/kg)	Change in standard bicarbonate (m-equiv/litre ± s.e.)	Change in pH (pH ± s.e.)	Change in pCO_2 (mm Hg ± s.e.)
0·25 (mixed group)	−0·2 ± 0·3	−0·027 ± 0·005	+4·3 ± 0·8
0·5 (male group)	−0·9 ± 0·3	−0·070 ± 0·012	+11·6 ± 1·4
0·5 (female group)	+0·2 ± 0·8	−0·071 ± 0·015	+13·9 ± 1·4
1·0 (male group)	−5·6 ± 0·9	−0·201 ± 0·023	+18·6 ± 3·1
1·0 (female group)	−5·1 ± 1·6	−0·209 ± 0·043	+17·7 ± 6·8
2·0 (mixed group)	−9·1 ± 0·9	−0·310 ± 0·027	+31·3 ± 6·1

* Note: the changes in standard bicarbonate and pCO_2 concurrent with the maximum change in pH are not necessarily the maximum changes themselves.

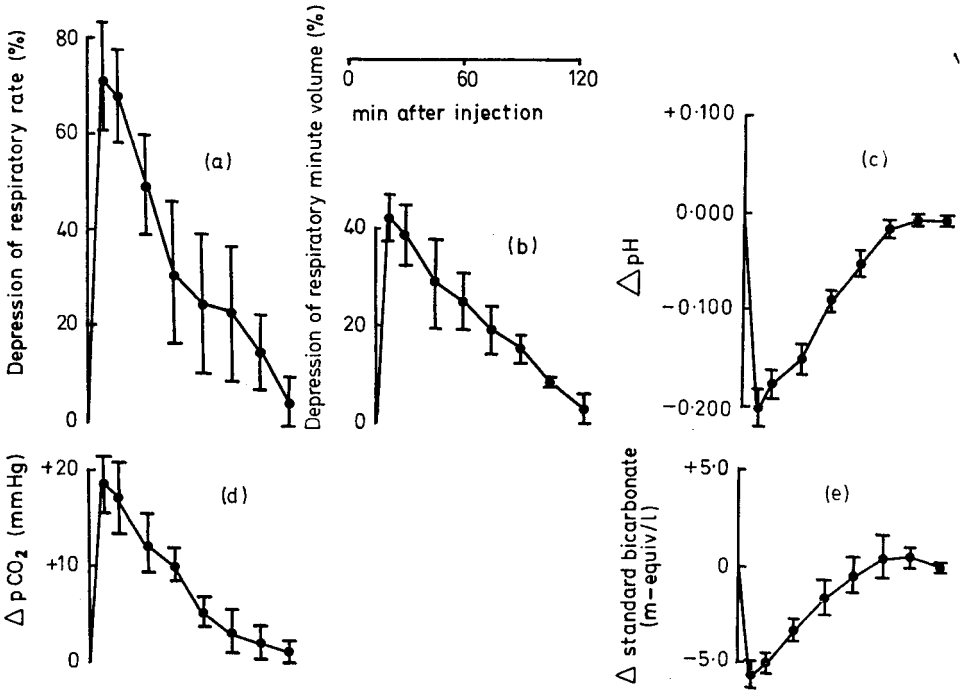

FIG. 5. The time course of the effect of 1 μg/kg etorphine in a group of four male rabbits on respiratory rate (a), minute volume (b), pH (c), blood pCO_2 (d) and standard bicarbonate (e). Means are ± standard error.

The maximum change in pH with the concurrent values for standard bicarbonate are shown in Table 4. At doses of 1 and 2 μg/kg there was a marked fall in bicarbonate associated with a severe acidosis.

Effects of etorphine—time course. A full record of changes in minute volume, rate, pH, pCO_2 and standard bicarbonate is shown for one of the groups (male group given 1 μg/kg) in Fig. 5. The important features are the rapid onset and short duration of effect, the relatively small change in minute volume compared with that of rate and the severe fall in pH.

Effects of etorphine—sex differences. Eight rabbits were investigated after doses of 0·5 and 1·0 μg/kg in order to identify any sex differences. There was no significant difference between the changes in standard bicarbonate observed in the two sexes.

Two of the female rabbits given 0·5 μg/kg gave anomalous results with respect to all parameters. Later it transpired that they were in the later stages of pregnancy at the time of the experiment. The effects in these two animals are of interest. The mean maximum depression of rate ($\pm$ s.e.) in the group of normal females was 42·1 $\pm$ 4·9%. The corresponding changes in the two pregnant animals represented a 32 and 66% *increase* in respiratory rate. Whilst there was a 12% increase in tidal volume in the normal group there was a 33 and 44% *decrease* in the pregnant animals. The elevation of pCO_2 and depression of minute volume in the two pregnant animals were both substantially less than those obtained from the normal group.

Effects of morphine on respiration. The effects of 2, 4 and 8 mg/kg of morphine on respiratory rate and minute volume are shown in Fig. 6. These changes should be compared with the parallel changes produced by etorphine (Fig. 4).

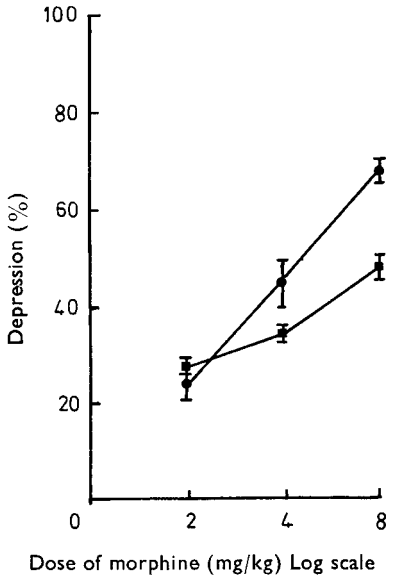

FIG. 6. The relation between the dose of morphine and the percentage depression of respiratory rate (circles) and respiratory minute volume (squares) in groups of rabbits. Means are $\pm$ standard error.

Effects of inhaled gases in animals treated with morphine and etorphine. Inhalation of 100% O_2 did not significantly alter the changes in minute volume produced by 4 mg/kg of morphine or 1 μg/kg of etorphine.

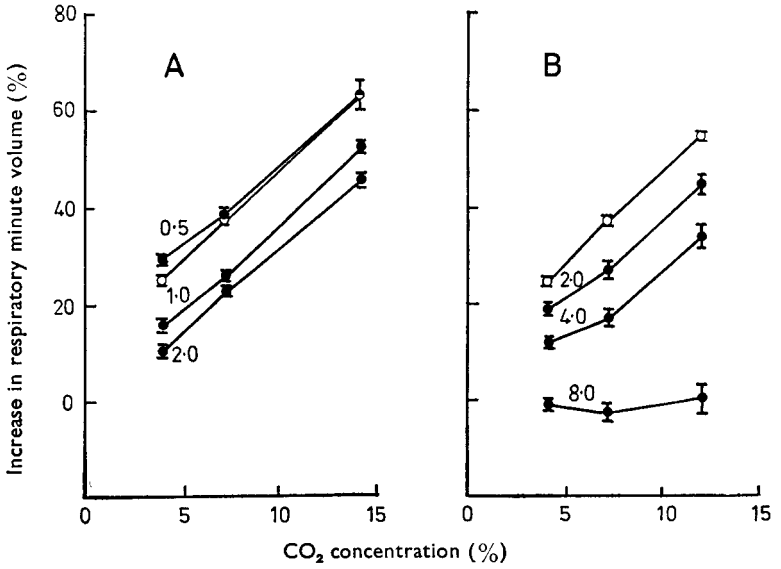

FIG. 7. The percentage increase in respiratory minute volume during inhalation of carbon dioxide in groups of rabbits treated with etorphine or morphine. Open circles are the control readings, closed circles show the values after drug treatment. The figures above each line refer to the dose of A, etorphine (μg/kg) and B, morphine (mg/kg). Means are $\pm$ standard error.

Doses of 2 and 4 mg/kg of morphine produced a parallel shift in the CO_2 concentration/respiratory minute volume slope (Fig. 7). A dose of 8 mg/kg completely abolished the stimulant action of CO_2 on minute volume up to concentrations of 12%.

On the other hand, when animals treated with etorphine were exposed to CO_2 a quantitatively different response was seen (Fig. 7). In animals given 0·5 μg/kg of etorphine there was no depression of CO_2 sensitivity up to concentrations of 14%. Indeed, there was a significant *increase* in sensitivity to 4% CO_2. Doses of 1 and 2 μg/kg produced a parallel shift of the CO_2 concentration/minute volume slope.

DISCUSSION

The Gaddum respirometer measures inspiratory flow rate by recording the pressure drop across a capillary tube which is acting as a resistance to inspiratory flow. It was possible that the inclusion of this resistance would place an inspiratory load upon the animal, causing a change in respiration which might not remain constant after drug treatment. To ensure that this did not influence our own work three different resistances were used in some experiments. The calibration line for each resistance is shown in Fig. 1. All gave a linear relation over the minute volume ranges encountered in this study. If the introduction of a capillary resistance into the inspiratory flow were to produce an abnormal respiratory pattern the extent of the abnormality should be related to the bore of the capillary. Table 1 shows that when each resistance was used during six different experimental types there was no significant difference between the percentage changes in minute volume determined with each resistance, nor was there a trend in the values related to capillary bore. It is concluded that the capillary resistance does not influence drug-induced changes in respiration.

The capillary resistance used routinely had an internal diameter of 2 mm—that originally recommended by Gaddum (1941) for the rabbit.

The remarkable potency of etorphine as a narcotic analgesic under all test conditions is well known (Blane, Boura & others, 1967). In this report 0·72 μg/kg caused a 50% depression of respiratory rate in the rabbit. The dose of morphine needed to produce a 50% depression of rate in the rabbit was found to be 3·7 mg/kg (Hunter, Pleuvry & Rees, 1968) and therefore etorphine is about 5000 times more potent than morphine in the present test conditions.

The marked periodic breathing produced by higher doses of etorphine has frequently been reported for other narcotic analgesics. Breckenridge & Hoff (1952; 1953) have reported periodic breathing after both morphine and levorphanol and concluded that this was indicative of "pharmacologic decerebration" caused by inactivation of cortical and subcortical suppressor mechanisms normally impinging upon the respiratory centre. Orkin, Egge & Rovenstine (1955) observed that this phenomenon was prominent after intravenous injection and in patients with depressed cerebral function.

The mechanism by which narcotic analgesics depress respiration has yet to be fully established. The depressant action on the sensitivity of the respiratory centre to carbon dioxide and/or hydrogen ion seems to be of primary importance (Eckenhoff & Oech, 1960), but even this has been questioned (Krueger, 1955). It is evident that the primary target for etorphine's respiratory depressant activity lies amongst the factors controlling respiratory rate. In all instances there was an immediate and often striking increase in tidal volume and the percentage depression of minute volume was far less than that of rate.

Such a marked increase in tidal volume produced by etorphine is not a characteristic action of narcotic analgesics. Hunter & others (1968) investigated the intravenous effects of nine narcotic analgesics in the rabbit, concluding that three of these produced a slight decrease in tidal volume, two a slight increase and four had no effect. Doses of morphine up to 20 mg given by either the intravenous or intramuscular routes caused a depression of tidal volume (Loeschcke, Sweel & others, 1953; Dripps & Comroe, 1945; Huggins, Spencer & others, 1957; Eckenhoff, Helrich & others, 1955). Alphaprodine and pethidine have been shown to produce a persistent depression of tidal volume (Orkin & others, 1955).

To preclude the possibility that etorphine initially depressed tidal volume but that it had returned to normal or even increased by the time of our first measurements —a phenomena reported after intravenous morphine (Foldes, Swerdlow & Siker, 1964)—tidal volume changes were continuously investigated after injection of etorphine (Table 3). Our results clearly show that the increase in tidal volume was instantaneous.

Hunter & others (1968), who showed that narcotic analgesics had no consistent effect on tidal volume in the rabbit, used a cumulative method of drug administration. However, we could show that when etorphine was injected either as a single dose, or in divided doses the effects on the respiratory parameters were the same.

In the light of the relatively enormous changes in tidal volume after etorphine the rabbit's normal response to an elevated ambient CO_2 mixture is of considerable interest. With CO_2 mixtures up to 15% a dose-dependent increase in tidal volume was observed which was never accompanied by an increase in respiratory rate. It is not unreasonable to assume that this was a direct consequence of the increased

alveolar and therefore arterial pCO_2. The fact that this change in tidal volume still occurred when respiratory rate was depressed by etorphine could imply that depression of the CO_2-sensitive medullary chemoreceptors contributes less to the mechanism of etorphine's respiratory depression than it does to other narcotic analgesics.

This suggestion is supported by the effects of CO_2 in rabbits given morphine or etorphine. The effects of CO_2 inhalation after morphine were predictable (Bellville & Seed, 1960, and references cited therein). There was a dose-dependent depression of CO_2 sensitivity *which was concurrent* with respiratory depression. A dose of 8 mg/kg totally abolished the stimulatory effect of concentrations of CO_2 up to 12%. On the other hand, a dose of 0·5 μg/kg of etorphine which produced considerable respiratory depression did not depress CO_2 sensitivity. Indeed, there was a significant increase in sensitivity to 4% CO_2. Higher doses of etorphine depressed CO_2 sensitivity but this was always quantitatively less than that caused by equi-respiratory depressant doses of morphine. A dose of 2 μg/kg of etorphine, which produced over 90% depression of rate, depressed CO_2 sensitivity less than did 4 mg/kg of morphine—a dose which only depressed rate by 44%.

Rees (1967; 1968) examined the effects of narcotic analgesics on blood pCO_2. It was shown that for drugs with a long duration of action (morphine) the rise in pCO_2 was accompanied by an increase in standard bicarbonate. A short-acting drug (dextromoramide) showed no such change whilst a drug of intermediate duration (phenazocine) exhibited a sex hormone-dependent sex difference in which only the female animals showed an increase in bicarbonate. The absence of any increase in standard bicarbonate after etorphine is compatible with this earlier pattern since etorphine has a similar duration of action as dextromoramide.

The finding that etorphine causes an increase in respiratory rate in pregnant females, but a depression in normal females is of interest. A sex hormone imbalance in favour of progesterone will result in a shift to the left of the slope relating alveolar pCO_2 to minute volume (Doring, Loeschcke & Ochwadt, 1950). This effect is in direct contrast to that produced by morphine. It is possible therefore that in the presence of progesterone the effect of etorphine would be reduced.

This provides another possible involvement of steroids in the mechanism of action of the narcotic analgesics (Rees, 1968, and references cited therein). The narcotic analgesic receptor proposed by Beckett & Casy (1954) would seem to be an over-simplification. With the advent of the highly potent narcotic analgesics (typified by etorphine), the hydroxyl group on the C-19 is of considerable importance to the compound's pharmacological activity and an additional part of the receptor must be considered (Leadbeater & Davis, 1968; Harris & Dewey, 1967).

We consider that the stereochemical similarities between the steroidal hormones and the most potent of the narcotic analgesics are too striking for their interactions to be dismissed as being coincidental. This is of interest in view of the recent finding by Craig (1968) that a dimethyl-aminomethyl substituted steroid possesses analgesic activity, causes a Straub tail in mice, reduces gastrointestinal propulsion and depresses respiration in rabbits—an effect which may be antagonized by nalorphine.

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