Br. J. Pharmac. Chemother. (1967), **30**, 11–22.

ACTIONS OF ETORPHINE HYDROCHLORIDE, (M99): A POTENT MORPHINE-LIKE AGENT

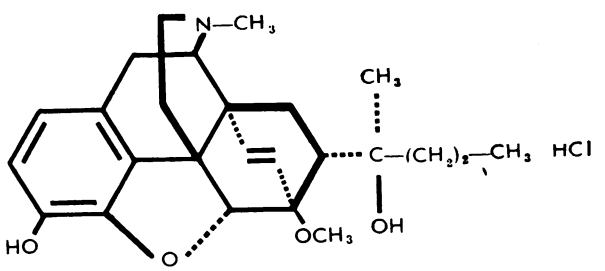
BY

G. F. BLANE, A. L. A. BOURA, A. E. FITZGERALD AND R. E. LISTER*

From the Pharmacology Laboratory, Reckitt & Sons, Hull

(*Received August* 16, 1966)

Etorphine hydrochloride (M99 Reckitt), is one of the more active of a number of derivatives of 6,14-*endo*ethenotetrahydro-oripavine, synthesized by Bentley & Hardy (1963) and briefly reported by Lister (1964) to possess powerful narcotic properties. When given subcutaneously etorphine is 1,000–80,000 times more potent than morphine, depending on the test situation, and its ability to cause catatonia at very low dose levels has resulted in its use for immobilizing game animals (J. R. Condy, cited by Sugden, 1964; Harthoorn, 1965a, b; Harthoorn & Bligh, 1965; King & Carter, 1965; King & Klingel, 1965). This paper extends the original work by describing in more detail the effects of the drug in laboratory species.



Etorphine hydrochloride; (propylorvinol hydrochloride; M99 Reckitt); 7α-(1-(*R*)-hydroxy-1-methylbutyl)-6,14-*endo*ethenotetrahydro-oripavine hydrochloride.

METHODS

Many of the methods employed were identical to those described in greater detail by Boura & Fitzgerald (1966) for the investigation of M320, N-(cyclopropylmethyl)-19-isopentylnororvinol hydrochloride.

Central nervous system

Analgesia. This was measured in mice (C.D. strain) using application of heat to the tail as the nociceptive stimulus (Grotto, Dikstein & Sulman, 1965). Groups of 10 male animals each received one of a logarithmic series of doses of the drug before having their tails immersed in water at 55° C. Those animals failing to withdraw their tails from the water within 5 sec were regarded as showing analgesia. The ED50 was calculated from the per cent failing to respond at each dose level.

* Present address: Arthur D. Little Ltd., Musselburgh, Scotland.

In male rats (C.F.E. strain) analgesia was assessed by the tail pressure method of Green & Young (1951). An estimate of the relative durations of the analgesic actions of etorphine and morphine in this species was obtained by determining the time necessary for the effect of a subcutaneous dose causing analgesia in 80% of the animals tested to fall to a level causing analgesia in 20%.

In the guinea-pig the multiple toe-pinch test, described by Collier, Warner & Skerry (1961) was employed to measure analgesia.

Pain thresholds in dogs and monkeys were determined using a modified form of the apparatus described by Green (1953).

Catatonia and respiratory depression. These effects were measured in the rat and guinea-pig using methods similar to those previously described (Boura & Fitzgerald, 1966). Catatonia was considered to be present if both hind feet were not removed within 45 sec when placed in turn on a metal rod 3 cm above bench level. Respiratory depression was assessed by determining the dose of drug required to depress the frequency of respiratory movements by 40%.

Conditioned avoidance studies. The effects of drugs on a conditioned avoidance-escape response developed by female rats (C.F.E. strain) were studied using the pole-climbing technique described by Cook & Weidley (1957). Rats were conditioned to avoid an electric shock after receiving an auditory stimulus, by placing each animal several times a day in a box the floor of which consisted of a steel grid to which an intermittent electric potential (300 V at 5 pulses/sec) was applied concurrently with activation of a buzzer for 30 sec. The shock could be avoided if the rat climbed on to an insulated pole suspended vertically in the centre of the box. After approximately five days of this treatment the auditory stimulus was given alone and, if necessary, reinforced after 10 sec by administration of the electrical stimulus. This routine established a conditioned reflex in all the rats tested by the tenth day ; each animal responding to the buzzer by climbing on to the pole within 7 sec. Immediately before administration of drugs establishment of the reflex was verified by activating the buzzer alone, following which each animal was placed on the grid and reinforced by simultaneous application of the auditory and electrical stimuli. Groups of 10 were injected subcutaneously with one of a logarithmic series of doses of the drug under test, 30 min before testing for presence of the conditioned and unconditioned responses. Exposure to the auditory stimulus alone was immediately followed by simultaneous application of the auditory and electrical stimuli. ED50s for inhibition of the conditioned reflex and the unconditioned reflex were calculated from the % in each group failing to climb on to the pole within 7 sec in response to the auditory and electrical stimuli respectively.

Spinal reflexes. The effects of etorphine on spinal reflexes were investigated in cats whose central nervous systems cephalad to C1 had been destroyed during a short period of ether anaesthesia by Dale's method as described by Burn (1952). The reflexes were elicited using the techniques described by Liddell & Sherrington (1929) and Schweitzer & Wright (1937). The crossed extensor reflex was obtained by stimulating the central end of the left sciatic nerve while recording the tension developed in the contralateral quadriceps femoris muscle, and the flexor reflex by stimulating the central end of the left popliteal nerve and recording contractions of the ipsilateral tibialis anticus muscle. The patella reflex was obtained by striking the right patella tendon at intervals, utilizing a Schweitzer-Wright hammer, after flexing the knee and recording movements of the tibia. Effects of etorphine on inhibition of the patella reflex caused by stimulating the central end of the ipsilateral sciatic nerve were also studied. All nerves were severed, placed on shielded bipolar platinum electrodes and stimulated for 5 sec every 2 min with various frequencies of rectangular pulses of 0.2 msec duration. The resulting muscular contractions were recorded isometrically using a strain gauge, mounted on a Brown-Schuster myograph stand, connected to a Physiograph.

Behavioural changes. Groups of mice (C.D. strain), rats (S.D. strain), cats, Beagle dogs, Green baboons (*Papio papio*), Squirrel monkeys (*Saimiri sciurea*) and Rhesus monkeys (*Macaca mulatta*) containing members of each sex were used. Rats and mice were caged in groups of three to five. Cats, dogs and primates were maintained singly in their home cages. Following administration of drugs they were observed continuously for 4 hr and at intervals for five days.

Activity of mice was recorded using the apparatus described by Dews (1953).

Cardiovascular and respiratory systems

Arterial blood pressure, heart rate and respiratory movements were recorded from anaesthetized dogs, Rhesus monkeys and cats, and unanaesthetized rats. Dogs and monkeys were anaesthetized with pentobarbitone sodium (36 mg/kg intravenously and 60 mg/kg intraperitoneally respectively). Anaesthesia in cats was induced with ether and maintained with intravenous chloralose (50 mg/kg). In the supine dog, cat and monkey arterial blood pressures were measured after cannulation of either the carotid or femoral arteries. In rats, restrained in a prone position, arterial blood pressures were recorded 3–24 hr after catheterizing the common carotid artery during a brief period of ether anaesthesia using a modification of the method described by Weeks & Jones (1960). In all four species pressure changes were recorded using Statham or Electronics and Medicine Inc. transducers and the electrocardiogram (lead II) used to trigger a cardiotachometer. Electrodes placed on opposite sides of the chest and connected to an impedance pneumograph facilitated recording the respiratory movements. All parameters were displayed on a multi-channel Physiograph. Drugs were injected subcutaneously into the dorsal cervical region of rats and into a cannula placed in a femoral vein of the other species.

Antagonism of etorphine and morphine

Antagonism of the analgesic actions of etorphine and morphine in the rat, by nalorphine and also M285, *N*-cyclopropylmethyl-7α-(1-hydroxy-1-methylethyl)-6,14-*endo*ethenotetrahydronororipavine a potent nalorphine-like agent (Bentley, Boura, Fitzgerald, Hardy, McCoubrey, Aikman & Lister, 1965), was studied using the tail pressure test. The experimental design was similar to that described by Cox & Weinstock (1964) for the quantitative study of nalorphine in the mouse.

The dose ratio (x), (Gaddum, Hameed, Hathway & Stephens, 1955), of the ED50 for the analgesic given alone and that obtained in the presence of the antagonist was determined. Log $(x-1)$ was plotted against the negative logarithm of the molar dose (per 100 g body wt.) of the antagonist. Finally, the pA_2 value for the antagonist, which may be obtained from the point of intersection of the regression line with the abscissa (Schild, 1947 ; Arunlakshana & Schild, 1959 ; Cox & Weinstock, 1964), and its standard error were calculated.

Gastrointestinal propulsion and urine excretion.

Inhibition of gastrointestinal propulsion in rats and guinea-pigs was measured using the techniques of Macht & Barba Gose (1931), Green (1959) and Boura & Fitzgerald (1966). Effects on urine excretion were studied in the rat using the method previously described (Boura & Fitzgerald, 1966).

Acute toxicity

Acute toxicities were determined using the intravenous, subcutaneous and oral routes in male mice weighing 19–24 g (C.D. strain) and in male rats weighing 60–80 g (C.F.E. strain) using groups of 10–20. The animals were kept in an ambient temperature of 23° C and observed for 48 hr. Each group was injected with one of a logarithmic series of doses of the drug under test and from the mortalities observed the LD50s and limits of error were calculated.

Statistical examination of data

ED50s and LD50s, together with their 95% confidence limits, were determined by a method based on those described by Berkson (1953) and Finney (1962). After calculating the equation of the logit response line, initial estimates of these parameters were found by the method of minimum logit Chi-squared. These were improved by iterating towards a maximum likelihood solution.

Drugs

All doses are expressed as the weight of the salt used. Etorphine and M285 were administered as the hydrochlorides, morphine as the sulphate and nalorphine as the hydrobromide. Atropine sulphate and acetylpromazine acid maleate were also used. The molecular weights of etorphine base and morphine base are 411 and 285 respectively.

RESULTS

Central nervous system

Analgesia. After intravenous administration to mice and rats the analgesic action of etorphine developed more rapidly than that of morphine. ED50s for analgesia determined at various times after intravenous administration of either etorphine or morphine are shown in Table 1. The peak effect of the latter drug, indicated by the lowest ED50 value obtained, occurred 5–15 min after its administration whereas the ED50 for etorphine determined at 1 min was not significantly different from those determined 5 and 15 min after injection. In each test the slopes of the regression lines, relating log

TABLE 1

POTENCIES OF ETORPHINE HYDROCHLORIDE AND MORPHINE SULPHATE AS ANALGESICS, DETERMINED BY THE HEATED TAIL METHOD IN THE MOUSE AND BY THE TAIL PRESSURE METHOD IN THE RAT, AT VARIOUS TIMES AFTER INTRAVENOUS ADMINISTRATION

95% confidence limits are in parentheses

Species	Time after injection (min)	Morphine sulphate ED50 (mg/kg)	Etorphine hydrochloride ED50 (μg/kg)
Mouse	1	2·43 (1·4–4·2)	2·38 (1·3–4·2)
	5	0·27 (0·1–0·5)	1·77 (0·9–3·3)
	15	0·49 (0·2–1·0)	5·12 (2·2–11·9)
Rat	1	2·10 (1·6–2·8)	0·23 (0·1–0·4)
	5	0·40 (0·3–0·7)	0·21 (0·1–0·3)
	15	1·60 (1·1–2·4)	0·18 (0·1–0·3)

dose of each drug to its effect, did not differ significantly from parallelism ($P>0.05$). Comparison of the ED50s obtained at the time of peak effect of each drug shows that etorphine given intravenously is approximately 150 and 2,000 times more potent than morphine in the mouse and rat respectively.

Subcutaneously in rats etorphine was approximately 1,700 times more potent than morphine (Table 2) but after equianalgesic doses the duration of its action was similar; the time required for the effect of the subcutaneous ED80 to fall to a level equivalent to

TABLE 2

RELATIONSHIP BETWEEN DOSE LEVELS OF ETORPHINE AND MORPHINE REQUIRED TO CAUSE ANALGESIA, CATATONIA AND RESPIRATORY DEPRESSION 30 MIN AFTER SUBCUTANEOUS ADMINISTRATION TO RATS AND GUINEA-PIGS

95% confidence limits in parentheses. † ED40.

Test	Species	Morphine sulphate ED50 (mg/kg)	Morphine sulphate Activity relative to analgesic dose	Etorphine hydrochloride ED50 (μg/kg)	Etorphine hydrochloride Activity relative to analgesic dose
Analgesia	Rat	2·9 (2·4–3·5)	1·0	1·7 (1·3–2·1)	1·0
Catatonia		4·9 (3·4–7·0)	0·59	2·2 (0·8–5·9)	0·8
Respiratory depression†		12·1 (2·7–53·5)	0·24	3·45 (0·5–23·0)	0·49
Analgesia	Guinea-pig	8·6 (7·6–9·7)	1·0	1·0 (0·8–1·2)	1·0
Catatonia		16·0 (6·6–38·6)	0·54	0·2 (0·1–0·4)	5·0
Respiratory depression†		229 (14–3,864)	0·04	8·6 (1·1–65·3)	0·12

the ED20 was 45 min for etorphine and 38 min for morphine. High potency was also found using the guinea-pig, etorphine being 8,600 times more active than morphine following subcutaneous administration. In mice etorphine was approximately 850 times more potent than morphine when given by this route (Table 3).

Etorphine was much less active when administered orally to mice and rats (Table 3), as dose levels 200–400 times those necessary subcutaneously were required to cause an equivalent degree of analgesia. Morphine was relatively well absorbed when given by stomach tube in the rat, but in the mouse was no better than etorphine.

TABLE 3

POTENCIES OF ETORPHINE HYDROCHLORIDE AND MORPHINE SULPHATE AS ANALGESICS IN THE RAT AND MOUSE, 30 AND 60 MIN AFTER BEING GIVEN BY THE SUBCUTANEOUS AND ORAL ROUTES RESPECTIVELY

95% confidence limits are in parentheses

Species	Drug	Subcutaneous ED50 (mg/kg)	Per Os ED50 (mg/kg)	P.O./S.C.
Rat	Morphine sulphate	2·9 (2·4–3·5)	28·0 (13·3–58·8)	10
	Etorphine hydrochloride	0·0017 (0·0013–0·0021)	0·74 (0·51–1·2)	435
Mouse	Morphine sulphate	6·8 (4·5–10·2)	>1,000	>147
	Etorphine hydrochloride	0·008 (0·0036–0·018)	1·45 (0·8–1·81)	181

Catatonia and respiratory depression. In Table 2 the subcutaneous doses of etorphine and morphine causing catatonia and respiratory depression in the rat and guinea-pig are compared with those necessary to cause analgesia. Relative to its analgesic effect, etorphine caused in both species a greater degree of catatonia and respiratory depression than did morphine. Using the rat the slope of the regression line relating log dose of etorphine to its catatonic effect (0.7 ± 0.02) was less steep than that obtained using morphine (2.4 ± 0.03). In each of the other test situations the slopes of the two regression lines obtained were not significantly different ($P > 0.05$).

Conditioned avoidance studies. The results obtained are summarized in Table 4. Etorphine contrasted with morphine by inhibiting the unconditioned reflex at dose levels much closer to those required either to block the conditioned reflex or to cause analgesia.

TABLE 4

DOSES OF ETORPHINE HYDROCHLORIDE AND MORPHINE SULPHATE REQUIRED TO BLOCK CONDITIONED AND UNCONDITIONED REFLEXES BY 50% IN RATS

95% confidence limits in parentheses

Drug	Time of peak effect (min)	Dose (μg/kg s.c.) Conditioned reflex (C.R.)	Dose (μg/kg s.c.) Unconditioned reflex (U.R.)	UR/CR	CR/Analgesic ED50 (μg/kg)
Etorphine	15–30	2·8 (1·8–4·3)	12·0 (10·5–13·7)	4·3	1·6
Morphine	30–60	9,800 (7,900–12,100)	≃200,000	≃20	3·4

Spinal reflexes. Etorphine, when given intravenously in the cat, markedly depressed spinal polysynaptic reflexes (Fig. 1). The ease with which the reflexes were depressed was crossed extensor>inhibition of patella>flexor≫patella (Table 5). After-discharge was blocked by even lower doses of etorphine (0.03–0.06 μg/kg) than were required to block the crossed extensor. Analogous results were obtained using morphine (1–100 mg/kg). Relative to the dose causing analgesia in other species etorphine appeared to depress spinal reflexes in the cat more readily than morphine, but due to the small number of animals receiving the latter drug it was not possible to establish this difference as statistically significant.

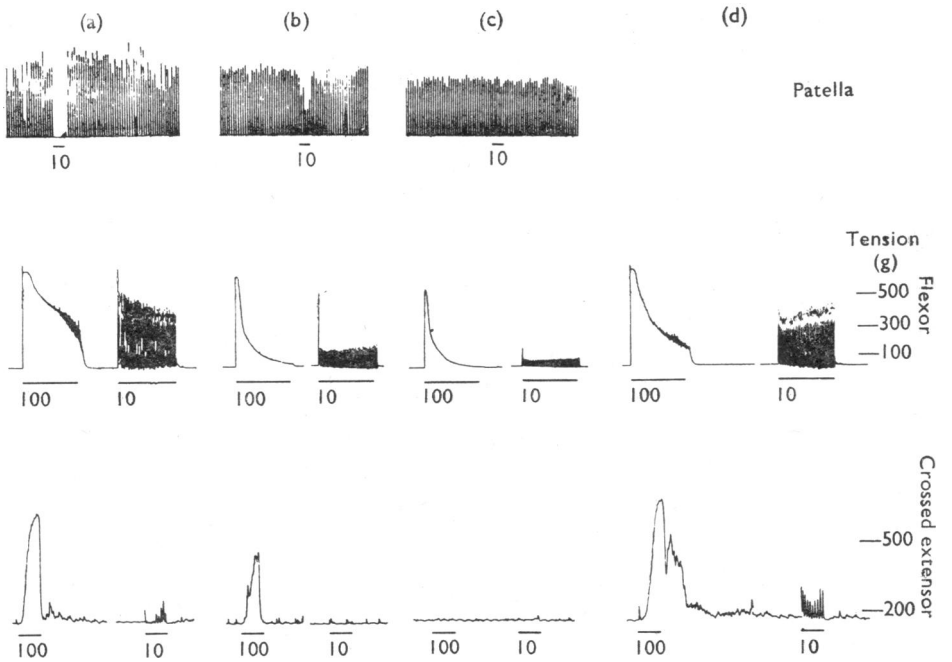


Fig. 1. Effects of etorphine on spinal reflexes of the cat. During the periods represented by the horizontal lines nerves were stimulated for 5 sec, the frequency of stimuli (pulses/sec) being indicated by the figure below each line. Upper tracing: inhibition of patella reflex caused by stimulating the ipsilateral sciatic nerve; (a)=control, (b)=after injection of 0.8 μg/kg etorphine. (c)=after injection of 6.5 mg/kg etorphine. Middle tracing: flexor reflex obtained by stimulating the ipsilateral popliteal nerve; (a)=control, (b) and (c) after injection of 6.4 μg/kg and 6.6 mg/kg etorphine respectively, (d)=after 30 mg/kg nalorphine. Lower tracing: crossed extensor reflex caused by stimulating the contralateral sciatic nerve; (a)=control, (b) and (c) after 0.06 μg/kg and 0.48 μg/kg etorphine respectively, (d)=after 5 mg/kg nalorphine.

Behavioural changes. In mice very small doses of etorphine given subcutaneously (3–10 μg/kg) caused a marked increase in locomotor activity, Straub tails and mydriasis. Loss of the pinna reflex occurred after these doses, but the corneal reflex persisted even after the administration of high dose levels (30–100 μg/kg). Ataxia and some depression of the righting reflex occurred after 3–10 μg/kg etorphine.

TABLE 5

DOSES OF ETORPHINE HYDROCHLORIDE REQUIRED TO CAUSE 80–100% INHIBITION OF SPINAL REFLEXES

Reflex	No. of cats	Frequency of stimulation (pulses/sec)	Mean Dose (μg/kg)$\pm$S.E.
Crossed extensor	4	10	0·28$\pm$0·07
		100	0·28$\pm$0·07
Inhibition of patella	2	10	4·7$\pm$3·2
Flexor	3	10	25·1$\pm$11·3
		100	29·9$\pm$9·2
Patella	3	0·1–1·0	>6,300

In rats subcutaneous doses of etorphine caused analgesia, sedation, respiratory depression and a morphine-like catatonic rigidity. Analogous effects were found in the guinea-pig (Table 2).

Excitement was the predominant effect seen after the subcutaneous administration of etorphine to cats. Slight hyperkinesis was detected in three cats that received 1 μg/kg. Larger doses (3–10 μg/kg) caused periods of excitement alternating with ones of depression. These effects were accompanied by mydriasis, together with ataxia attributable to hind limb weakness. In three further cats 30 μg/kg etorphine caused similar effects and additionally myoclonus. Generalized tonic, alternating with clonic, convulsions occurred after very high doses had been administered (100–300 μg/kg). Excitement, but not the analgesia, caused by etorphine (10–30 μg/kg) was either reduced or abolished by subcutaneous administration of acetylpromazine (3–10 mg/kg intraperitoneally) in six cats.

The predominant effect of etorphine given parenterally to dogs was the rapid development of catatonia associated with total analgesia. Thus the effects of the drug in nine animals, seen within 2 min of intravenous injection of 2–5 μg/kg and lasting 30 min–2 hr, were ataxia and miosis followed by deep narcosis during which surgical procedures could be performed. At the higher doses there was a degree of salivation, bradycardia and respiratory depression in most animals. Defaecation also occurred in some dogs. In contrast to etorphine, morphine in low intravenous doses produced violent excitement. In five of seven dogs receiving 2–4 mg/kg morphine this lasted 30–60 sec, whereas it did not occur in seven injected with 2–5 μg/kg etorphine. This difference between the two drugs is highly significant ($P<0.01$).

Intramuscular administration of etorphine (2.5–5 μg/kg) resulted in the development of a similar pattern of response to that described above for the intravenous route. The effects of the drug commenced within 10–15 min after injection and lasted for 45 min–2 hr.

When given subcutaneously etorphine contrasted markedly with morphine in the dog by not causing emesis except after very high dose levels. In a crossover experiment using groups of four, etorphine at dose levels of 0.3–9.0 μg/kg failed to cause emesis, whereas morphine given by the same route in equianalgesic doses (0.3–9.0 mg/kg) caused emesis in all the animals, the difference between the two drugs being very highly significant ($P<0.001$). Emesis occurred only after very high subcutaneous dose levels of etorphine (0.1–1.0 mg/kg) had been administered to two further dogs. Narcosis was

substantially slower in onset, lacking in intensity and more prolonged when etorphine was administered by the subcutaneous route. In addition, low subcutaneous doses of etorphine (0.3–1 μg/kg) initially caused tachycardia accompanied by mydriasis while very large doses (up to 1 mg/kg) caused tremors, myoclonus and cyanosis but with complete recovery after 27–43 hr. Effects of etorphine, given by this or the other routes, could be readily antagonized by administration of nalorphine (0.25–1 mg/kg).

Low doses of etorphine (3 μg/kg) given intravenously to baboons, Squirrel and Rhesus monkeys caused narcosis and analgesia. Increasing the dose level to 10 μg/kg caused complete prostration within 90 sec, periodic breathing, together with cyanosis. These effects were again antagonized by intravenous nalorphine (1 mg/kg).

Etorphine was very much less effective when given orally to Squirrel and Rhesus monkeys. Although large doses of the drug (300 μg/kg or more) were absorbed to some extent in some animals, others appeared unaffected.

Cardiovascular and respiratory systems

Etorphine given subcutaneously to groups of three unanaesthetized rats at dose levels of 6, 12 and 24 μg/kg caused bradycardia together with a fall in arterial blood pressure and slowing of the respiratory rate. These effects reached a maximum approximately 20 min after injection and lasted for 30 min to 1 hr. The slowing of the heart rate but not the vasodepressor effect caused by etorphine (24 μg/kg) was antagonized by atropine sulphate (10 mg/kg subcutaneously) in four other rats.

Similar cardiovascular and respiratory effects were caused by etorphine when it was given intravenously to anaesthetized cats. In three animals 0.1–5.0 μg/kg etorphine slowed the heart rate by nearly 50%, and caused a mean fall of 48 mm Hg in the mean arterial blood pressure which took 5–10 min to develop and lasted for over an hour. Artificial respiration was required in one of two cats shortly after 0.3 μg/kg intravenously, and in two of three cats that received 1 μg/kg.

The effect of etorphine on the cardiovascular and respiratory systems of the anaesthetized dog is shown in Fig. 2. Bradycardia together with sinus arrhythmia, lowering of the mean arterial blood pressure by a mean value of 33 mm Hg and decreased respiratory frequency occurred shortly after the intravenous administration of 1 μg/kg etorphine to nine animals. These effects took 1–10 min to develop fully and lasted for over 1 hr. The hypotension but not the bradycardia persisted in two dogs that had been atropinized (1 mg/kg intravenously). The cardiovascular effects after this dose of etorphine were absent in two of three further dogs previously vagotomized. Higher intravenous dose levels of etorphine (5–10 μg/kg) led to a further decrease in arterial blood pressure and complete cessation of respiratory movements. These effects of etorphine were antagonized by the morphine antagonists nalorphine (1 mg/kg) or M285 (5–50 μg/kg) given intravenously.

In five anaesthetized monkeys the cardiovascular actions of etorphine were found to be very similar qualitatively to those observed in the dog, cat and rat, although the monkey appeared to be slightly more sensitive to the drug. Respiration in the monkey was usually completely arrested by an intravenous dose of 1 μg/kg etorphine but it tended to recover spontaneously after 1–2 min.

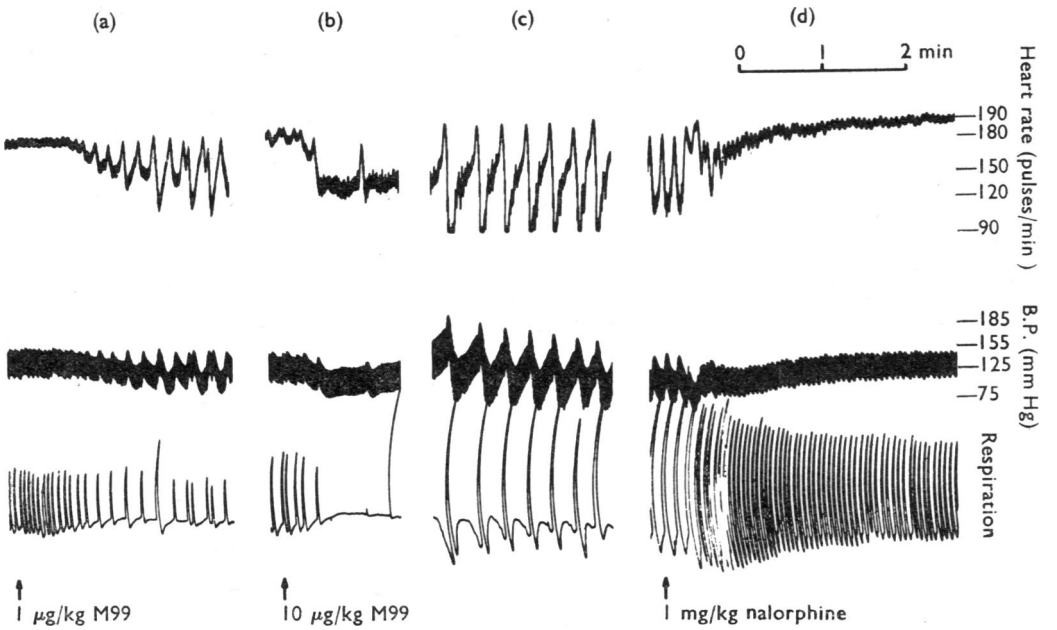


Fig. 2. Physiograph recording of the heart rate, arterial blood pressure and respiratory movements from a dog anaesthetized with pentobarbitone sodium. At the arrows 1 μg/kg etorphine, 10 μg/kg etorphine and 1 mg/kg nalorphine were injected intravenously. Between (a) and (b) 113 minutes elapsed, between (b) and (c) 18 min, between (c) and (d) 20 min.

Antagonism of etorphine and morphine

Both nalorphine and M285 competitively antagonized the analgesic action of etorphine in the rat. Arunlakshana & Schild (1959) have pointed out that competitive antagonism is demonstrated when the plot of log (x—1) against the negative log of N (where x is the ratio of the dose of agonist alone to that required to cause a similar effect in the presence of the molar concentration (N) of the antagonist) gives a linear regression. In our experiments (Table 6), the regression lines obtained using either antagonist did not differ significantly from linearity. The pA_2 value obtained for antagonism of etorphine, using either nalorphine or the highly potent M285, was not significantly different from that obtained for antagonism of morphine.

Gastrointestinal propulsion and urine excretion

Gastrointestinal motility in the rat and guinea-pig was reduced after subcutaneous administration of 0.05 μg/kg or more etorphine.

TABLE 6

pA_2 VALUES FOR NALORPHINE AND M285 AS ANTAGONISTS OF THE ANALGESIC ACTIONS OF MORPHINE AND ETORPHINE IN THE RAT

95% confidence limits are in parentheses

	Antagonist	
Analgesic	Nalorphine	M285
Morphine sulphate	5·6 (5·4–6·1)	8·2 (8·0–8·7)
Etorphine hydrochloride	5·5 (5·3–6·2)	8·2 (7·9–8·9)

Subcutaneous doses of 2–20 μg/kg etorphine in the water loaded rat caused a marked decrease in the volume of urine excreted. Morphine (10–100 mg/kg) given by the same route caused a similar effect.

Acute toxicity

Table 7 shows the acute toxicities of etorphine and morphine in mice and rats. Relative to its analgesic effect etorphine given to mice appeared to be considerably less toxic than morphine. Analogous findings were obtained giving the drugs intravenously to rats. The much smaller slopes of the regression lines relating log dose to mortality indicate that etorphine is relatively more toxic than morphine when given either subcutaneously or orally in low doses to the latter species.

TABLE 7

ACUTE TOXICITIES OF ETORPHINE HYDROCHLORIDE AND MORPHINE SULPHATE IN MICE AND RATS

95% confidence limits in parentheses

Compound	Species	Route	LD 50 (mg/kg)	Slope
Etorphine hydrochloride	Mouse	i.v.	80 (71–91)	7·0
		s.c.	425 (370–489)	9·1
		p.o.	1,856 (449–7,679)	1·42
Morphine sulphate	Mouse	i.v.	221 (188–260)	8·5
		s.c.	506 (445–575)	6·3
		p.o.	1,270 (1,095–1,472)	4·7
Etorphine hydrochloride	Rat	i.v.	5·3 (2·9–9·8)	1·23
		s.c.	53·4 (6·3–455·3)	0·5
		p.o.	71·9 (13·7–377·6)	0·4
Morphine sulphate	Rat	i.v.	100·0 (63·7–156·9)	1·5
		s.c.	170·0 (131–221)	3·0
		p.o.	461 (376–565)	4·38

DISCUSSION

The similarity that exists between the chemical structure of the highly potent etorphine and that of morphine is reflected in their pharmacological profiles with the implication that both drugs may exert their effects by actions on identical receptors. Support for this suggestion comes from the finding that the pA_2 values for nalorphine and M285 as antagonists of etorphine in the rat were not significantly different from those obtained for antagonism of morphine. Arunlakshana & Schild (1959) have suggested that two agonists act on the same receptor when they produce identical pA_x values with a competitive antagonist. Analogous conclusions were arrived at by Cox & Weinstock (1964) regarding the nature of the receptors mediating the analgesic and lenticular opacity producing effects of the 3 acetyl ester of etorphine (M183) in the mouse.

When given subcutaneously etorphine was 1,000–80,000 times more potent than morphine depending on the test situation and the species used. No complete dissociation of the various morphine-like effects examined could be demonstrated but interesting differences were nevertheless detected between the two drugs. Etorphine was found to depress to a relatively greater extent than morphine the lower centres of the central nervous system. Thus, etorphine caused greater respiratory depression in relation to

its analgesic effect in the rat and guinea-pig; and similar observations have been made using the rabbit (Leach, unpublished results). Similarly, catatonia and blockade of conditioned reflexes occurred in guinea-pigs and rats after administration of etorphine at dose levels very close to those necessary to cause analgesia and inhibit unconditioned reflexes. This was in marked contrast with the wide separation between the doses that were required of morphine. The greater prominence of the central depression after etorphine was further emphasized by findings that the drug blocked more readily spinal reflexes in the cat and failed to cause excitation and emesis in the dog. Another difference between the drugs was that the action of etorphine developed more rapidly after intravenous administration to mice and rats. Tolerance to the analgesic action of etorphine has been found to develop less rapidly than to that of morphine during daily administration of equianalgesic doses of the two drugs to rats (Ettles & Lister, in preparation).

The pharmacological actions of etorphine in laboratory animals are qualitatively similar to those of morphine but its uniquely high potency and more prominent central depression, which may be readily antagonized with nalorphine or M285, is consistent with expanding evidence of its value as an immobilizing agent for wild and domestic animals. Preliminary trials in human volunteers (Campbell & Lister, unpublished observations) confirm the laboratory findings that etorphine possesses powerful narcotic properties, but studies using the Rhesus monkey (Deneau & Seevers, 1964) indicate that there is no dissociation from physical dependence capacity.

SUMMARY

1. The effects of etorphine (Propylorvinol; M99, Reckitt), 7α-(1-(*R*)-hydroxy-1-methylbutyl)-6,14-*endo*ethenotetrahydro-oripavine, a powerful morphine-like agent, have been investigated and compared with those of morphine.

2. Given subcutaneously, etorphine was 1,000–80,000 times more potent than morphine depending on the test situation.

3. Etorphine resembled morphine in rodents, cats, dogs and monkeys by causing analgesia, catatonia, blockade of conditioned reflexes, respiratory depression, reduction of gastro-intestinal propulsion and an anti-diuretic effect. It also resembled morphine by causing excitement in mice and cats, and bradycardia and hypotension in rats, dogs, cats and monkeys.

4. Etorphine depressed the lower centres of the central nervous system to a relatively greater extent than morphine. The drug caused greater respiratory depression in rats, guinea-pigs and rabbits. It also blocked more readily conditioned reflexes in the rat and caused a greater degree of catatonia in the rat and guinea-pig. In contrast to morphine low doses of etorphine failed to cause excitement and emesis in the dog.

5. Nalorphine and the potent morphine antagonist M285 fulfilled conventional criteria for competitive antagonism of etorphine.

We are very grateful for the help given by Miss E. C. Leach and Miss P. J. Prince, and the technical assistance provided by Mr. E. J. R. Harry, Mr. I. R. Macfarlane, Miss J. Allison, Miss E. Moore, and Mr. G. Reed. Mr. C. Holden kindly carried out the statistical examination of our results. We should also like to thank Dr. G. F. Somers for kindly commenting on the manuscript. Secretarial assistance by Miss G. Cray is gratefully acknowledged.

REFERENCES

ARUNLAKSHANA, O. & SCHILD, H. O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

BENTLEY, K. W., BOURA, A. L. A., FITZGERALD, A. E., HARDY, D. G., MCCOUBREY, A., AIKMAN, M. L. & LISTER, R. E. (1965). Compounds possessing morphine-antagonizing or powerful analgesic properties. *Nature, Lond.*, **206**, 102–103.

BENTLEY, K. W. & HARDY, D. G. (1963). New potent analgesics in the morphine series. *Proc. chem. Soc.*, p. 220.

BERKSON, J. (1953). A statistically precise and relatively simple method of estimating the bioassay with quantal response, based on the logistic function. *J. Am. statist. Ass.*, **48**, 565–599.

BOURA, A. L. A. & FITZGERALD, A. E. (1966). The pharmacology of N-(cyclopropylmethyl)-19-isopentylnororvinol hydrochloride. A potent and long lasting central depressant. *Br. J. Pharmac. Chemother.*, **26**, 307–321.

BURN, J. H. (1952). *Practical Pharmacology*. Blackwell, Oxford.

COLLIER, H. O. J., WARNER, B. T. & SKERRY, R. (1961). Multiple toepinch method for testing analgesic drugs. *Br. J. Pharmac. Chemother.*, **17**, 28–40.

COOK, L. & WEIDLEY, E. (1957). Behavioral effects of some psychopharmacological agents. *Ann. N.Y Acad. Sci.*, **66**, 740–752.

COX, B. M. & WEINSTOCK, M. (1964). Quantitative studies of the antagonism by nalorphine of some of the actions of morphine-like analgesic drugs. *Br. J. Pharmac. Chemother.*, **22**, 289–300.

DENEAU, G. A. & SEEVERS, M. H. (1964). Evaluation of new compounds for morphine-like physical dependance in the Rhesus monkey. *Bulletin, Drug Addiction and Narcotics*, Addendum 1, 1.

DEWS, P. B. (1953). The measurement of the influence of drugs on voluntary activity in mice. *Br. J. Pharmac. Chemother.*, **8**, 46–48.

FINNEY, D. J. (1962). *Probit Analysis*. 2nd ed. University Press, Cambridge.

GADDUM, J. H., HAMEED, K. A., HATHWAY, D. E. & STEPHENS, F. F. (1955). Quantitative studies of antagonists for 5-hydroxytryptamine. *Q. J. exp. Physiol.*, **40**, 49–74.

GREEN, A. F. (1953). Analgesic and other properties of 3 : 3-dithienylalkenylamines. *Br. J. Pharmac. Chemother.*, **8**, 2–9.

GREEN, A. F. (1959). Comparative effects of analgesics on pain threshold, respiratory frequency and gastrointestinal propulsion. *Br. J. Pharmac. Chemother.*, **14**, 26–34.

GREEN, A. F. & YOUNG, P. A. (1951). A comparison of heat and pressure analgesiometric methods in rats. *Br. J. Pharmac. Chemother.*, **6**, 572–585.

GROTTO, M., DIKSTEIN, S. & SULMAN, F. G. (1965). Additive and augmentative synergism between analgesic drugs. *Archs. int. Pharmacodyn. Ther.*, **155**, 365–372.

HARTHOORN, A. M. (1965a). The use of a new oripavine derivative for restraint of domestic hoofed animals *J. S. Afr. vet. med. Ass.*, **36**, 45–50.

HARTHOORN, A. M. (1965b). Application of pharmacological and physiological principles in restraint of wild animals. *Wildl. Monogr., Chestertown*, No. 14.

HARTHOOPN, A. M. & BLIGH, J. (1965). The use of a new oripavine derivative with potent morphine-like activity for the restraint of hoofed wild animals. *Res. vet. Sci.*, **6**, 290–299.

KING, J. M. & CARTER, B. H. (1965). The use of the oripavine derivative M99 for the immobilization of the black rhinoceros (Diceros Bicornis) and its antagonism with the related compound M285 or nalorphine. *East African Wildlife Journal*, **3**, 19–26.

KING, J. M. & KLINGEL, H. (1965). The use of the oripavine derivative M99 for the restraint of equine animals and its antagonism with the related compound M285. *Res. vet. Sci.*, **6**, 447–455.

LIDDELL, E. G. T. & SHERRINGTON, C. (1929). *Mammalian Physiology*. 2nd ed. Clarendon Press, Oxford.

LISTER, R. E. (1964). Structure-activity requirements in some novel thebaine-derived analgesics. *J. Pharm. Pharmac.*, **16**, 364–366.

MACHT, D. I. & BARBA-GOSE, J. (1931). Two new methods for pharmacological comparison of insoluble purgatives. *J. Am. Pharm. Ass. sci. Ed.*, **20**, 558–564.

SCHILD, H. O. (1947). pA, A new scale for the measurement of drug antagonism. *Br. J. Pharmac. Chemother.*, **2**, 189–206.

SCHWEITZER, A. & WRIGHT, S. (1937). Effects on the knee jerk of stimulation of the central end of the vagus and of various changes in the circulation and respiration. *J. Physiol.*, **88**, 459–475.

SUGDEN, B. S. (1964). Veterinary Congress Discussion. *Vet. Rec.*, **76**, 1247–1248.

WEEKS, J. R. & JONES, J. A. (1960). Routine direct measurement of arterial pressure in unanesthetized rats. *Proc. Soc. exp. Biol. Med.*, **104**, 646–648.