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THE PHARMACOLOGY OF *N*-(CYCLOPROPYLMETHYL)-19-ISOPENTYLNORORVINOL HYDROCHLORIDE. A POTENT AND LONG LASTING CENTRAL DEPRESSANT

BY

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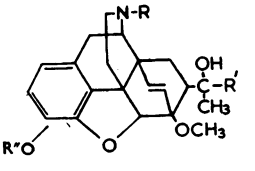
M320 [Reckitt ; *N*-(cyclopropylmethyl)-19-isopentylnororvinol hydrochloride] is one of a series of derivatives of 6, 14-*endo*ethenotetrahydro-oripavine reported by Bentley, Boura, Fitzgerald, Hardy, McCoubrey, Aikman & Lister (1965) to be powerful and long lasting analgesics in laboratory animals. Chemically it is related to morphine and also to M99 and M183 which recently have been shown to possess unprecedented potency as analgesics, both in laboratory animals (Bentley & Hardy, 1963 ; Lister, 1964) and man (A. H. B. Masson, cited by Campbell, Lister & McNicol, 1964). Following the finding of strong central depression caused by M320 in the rat by Aikman & Lister (unpublished), we have studied its actions in more detail and describe here some of the acute pharmacological effects of the drug and compare its properties with those of morphine.



M99: $R = CH_3$, $R' = [CH_2]_2.CH_3$, $R'' = H$

M183: $R = CH_3$, $R' = [CH_2]_2.CH_3$, $R'' = CH_3.CO$

M320: $R = CH_2—CH(CH_2)_2$, $R' = [CH_2]_2.CH(CH_3)_2$, $R'' = H$

METHODS

Central nervous system

Analgesia. This was assessed by the method of Hendershot & Forsaith (1959) in female mice (CD strain) weighing 17 to 23 g. Drugs at ascending dose levels, or saline for controls, were administered subcutaneously into groups of five mice, at various times before an intraperitoneal injection of phenyl-*p*-benzoquinone (2 mg/kg). The dose of the drug required to reduce by 50% the number of abdominal stretches, caused by the irritant action of the phenyl-*p*-benzoquinone, was calculated by comparison with the controls.

Analgesic activity in rats was determined using the tail pressure method described by Green & Young (1951). Male Sprague-Dawley rats weighing 80 to 120 g received parenterally either saline

as a control or one of a logarithmic series of doses of the drug under test, at various intervals of time before determining pain thresholds. The animals were regarded as showing analgesia if they failed to squeal on application of a pressure greater than twice the mean pressure required to cause a vocal response in the controls. From the percentage showing analgesia at each dose level the ED50 was calculated.

An estimate of the relative durations of analgesia following parenteral administration of either M320 or morphine in rats was obtained by determining from the time of peak action, the time necessary for the effect of a dose causing analgesia in 80% of the animals tested to fall to a level causing analgesia in 20%.

Development of tolerance to the analgesic actions of M320 and morphine was studied in groups of ten rats receiving daily single subcutaneous doses of either drug. The percentage showing analgesia in each group was recorded each day 30 min after administration of either three or ten times the ED50.

The multiple toe-pinch test described by Collier, Warner & Skerry (1961) was used to measure analgesia in guinea-pigs. Groups of ten animals weighing 250 to 450 g were used. A bulldog artery clip was applied for 2 to 3 sec to each of the fourteen toes of each animal in random order. The number of times the animal responded, by squeaking, after each application of the clip was recorded shortly before and 30 min after subcutaneous administration of drugs. The dose was calculated that caused a 50% reduction in the number of vocal responses.

Pain thresholds in Beagle dogs, weighing approximately 8 kg, were determined using a modification of the apparatus described by Green (1953). The apex of a triangular wedge fitted to the handle of the metal piston of a hypodermic syringe was applied to the dorsal surface of each paw and the pressure gradually increased until the animal responded by yelping or removing its paw. The air pressure inside the syringe necessary to cause this effect was measured by means of a Bourdon gauge connected to the syringe head. This was carried out first for control purposes and then at various intervals after parenteral administration of drugs.

Catatonia. The test was carried out, using groups of ten guinea-pigs or rats, shortly before and 30 min after the injection of the drug. Each hind-foot was placed in turn on a horizontal metal rod 3 cm above bench level and the animal was considered to be catatonic if neither foot was removed within 45 sec. Drugs were given subcutaneously, at ascending dose levels, and from the percentage showing catatonia in each group the ED50 was calculated.

Respiratory depression. In the guinea-pig and rat this was estimated by measuring the frequency of respiratory movements in groups of ten to twelve animals that had received subcutaneously 30 min earlier either the drug or saline. Respiratory movements were recorded by placing the flank of each animal for 20 sec against a large tambour connected by rubber tubing to a second tambour writing on a smoked drug. The dose required to depress the respiratory frequency by 40% was calculated by comparison with the controls.

Behavioural changes. Groups of mice, rats, cats, dogs and monkeys, containing members of either sex, were used. After subcutaneous administration of drugs the animals were observed continuously for 4 hr and, then, at intervals, for 5 days and overt changes were recorded.

Effects of drugs on the co-ordinated locomotor activity of mice were studied, using animals whose diurnal variation in activity had been transposed by reversing the normal sequence of lighting of their environment. The number of interruptions of an infrared beam by groups of three mice were recorded every 15 min using the apparatus described by Dews (1953). Counting commenced 30 min after subcutaneous injection of drugs or saline, and continued for 90 min.

Gastrointestinal tract

Decreased gastrointestinal propulsion. This was estimated by measuring the rate of passage of a test meal from the stomach along the small intestine of rats and guinea-pigs. The charcoal meal test, described by Macht & Barba-Gose (1931) and Green (1959), was used in the rat and modified for use in the guinea-pig. Both species were deprived of food for 18 to 24 hr but allowed water until 1 hr before the test. Groups of ten to twelve were injected subcutaneously with either the drug under test or saline 30 min before administration of the meal by stomach tube. Rats received

5 ml./kg of the charcoal meal and were killed 15 min later. Guinea-pigs received 10 ml./kg of a 66% barium sulphate suspension in distilled water and were killed 12 min later. In both species the abdomen was opened immediately after death and a haemostat was applied to the small intestine at the point reached by the leading edge of the meal. After removal of the stomach and small intestine, the distance travelled by the meal along the small intestine from the pyloric sphincter was measured and expressed as a percentage of the total length. The mean percentage travelled in each drug-treated group was expressed as a percentage of the mean travel for the control group. This final value was plotted against the logarithm of the dose administered and the dose was calculated that was required to reduce gastrointestinal propulsion by 50%.

Peristaltic reflex. Effects on the peristaltic reflex in the guinea-pig isolated ileum were studied by Trendelenburg's (1917) technique, using a modified Tyrode solution having a high calcium (0.4 g/l. calcium chloride) and low magnesium (0.03 g/l. magnesium chloride) content. The solution was oxygenated and maintained at 37° C. The threshold intraluminal pressure necessary to elicit the reflex was established first. Subsequently, for 1 min every 3 min, the pressure was changed from the subliminal by one of a series of progressive increases, up to a maximum of 4 cm of water above threshold. The responses to different pressures were determined once before and twice after adding a drug to produce one of a cumulative series of bath concentrations of the compound.

Urinary system

Effects on urine excretion were studied using groups of five male rats. They were deprived of food overnight but allowed water *ad libitum* until 1 hr before the test. For diuretic studies each animal received by stomach tube a small load of 0.9% saline (1 ml./100 g) together with a subcutaneous injection of saline or one of a logarithmic series of doses of the drug. Each group was placed in a metabolism cage and the urinary output was recorded every 30 min for 4 hr. For antidiuretic studies the same experimental technique was used with the exception that the animals received orally a water load of 5 ml./100 g.

Electrolyte excretion was determined by flame photometric analysis of the urine samples.

Acute toxicity

Acute toxicities were determined by the intravenous and subcutaneous routes in mice weighing 19 to 24 g (CD strain) and in rats weighing 60 to 80 g (C.F.E. strain) using groups of ten consisting of equal numbers of each sex. The animals were kept in an ambient temperature of 23° C and observed for 24 hr. From the mortalities observed the LD50s and limits of error were calculated.

Cardiovascular and respiratory systems

Effects of M320 on the arterial blood pressure, heart rate and respiration were studied in anaesthetized dogs, monkeys and cats. Dogs and monkeys were anaesthetized with pentobarbitone sodium (36 and 60 mg/kg respectively), given either intravenously or intraperitoneally. Anaesthesia in cats was induced with ether and maintained with intravenous chloralose (50 mg/kg).

Electrocardiograms (lead II) were monitored and used to trigger a cardiotachometer. Arterial blood pressures were measured by means of a pressure transducer connected to the carotid or femoral artery. Respiratory movements were recorded using electrodes, placed on opposite sides of the chest, connected to an impedance pneumograph. All parameters were displayed on a multichannel Physiograph. Drugs were injected into a cannula inserted into the central end of a femoral vein.

Antagonism of M320

Analgesia. Antagonism by nalorphine of the analgesia caused by M320 in the rat was investigated using groups of ten. Each group received subcutaneously one of a logarithmic series of doses of M320, and was injected either at the same time or 2.5 hr later by the same route either with saline or with one of a series of doses of nalorphine. The number showing analgesia in each group was determined by the method described above, 30 min after administration of the antagonist.

Respiratory depression. Antagonism of the reduction in respiratory minute volume caused either by M320 or by morphine was looked for in groups of four dogs using the apparatus described by Gaddum (1941). The analgesic was gradually administered intravenously until the animal was prostrate. Each dog was then connected to the apparatus by means of a closely fitting face mask and gradually increasing amounts of the antagonist were administered through a fine syringe needle placed in the anterior brachiocephalic vein, the ventilatory rate being recorded until recovery or for at least 2 hr. Approximately 3 weeks later the test was repeated except that M320 was administered to the animals that had previously received morphine and *vice versa.*

Drugs

All doses are expressed as the weight of the salt used. M320 was administered as the hydrochloride, morphine as the sulphate, nalorphine as the hydrobromide and levallorphan as the tartrate. Amiphenazole and bemegride were also used. The approximate molecular weights of M320 base and morphine base are 479 and 385 respectively.

RESULTS

Central nervous system

Analgesia. In mice M320 reduced the number of abdominal stretches caused by the intraperitoneal injection of phenyl-*p*-benzoquinone. Table 1 shows the doses of morphine and M320 required to inhibit the number of abdominal stretches by 50%. The action of M320 developed more slowly and persisted longer than that of morphine. Thus, the peak effect of M320 occurred 1 to 3 hr after its administration, indicated by the lower ED50 values at these times than at 30 min; whereas the ED50s for morphine at 1 and 3 hr were significantly greater than that determined 30 min after injection. At the time

TABLE 1

POTENCIES OF M320 HYDROCHLORIDE AND MORPHINE SULPHATE AS ANALGESICS IN THE MOUSE, AT VARIOUS TIMES AFTER SUBCUTANEOUS ADMINISTRATION

95% Confidence limits are in parentheses, and slopes include the ± standard errors

Time after injection (min)	M320 hydrochloride		Morphine sulphate	
	ED50 (μg/kg)	Slope	ED50 (μg/kg)	Slope
30	12·3 (10·6–14·4)	2·284±0·270	520 (368–754)	0·808±0·117
60	6·9 (5·8–8·2)	1·758±0·167	1,357 (1,124–1,639)	1·748±0·220
180	8·0 (6·9–9·3)	2·106±0·223	6,515 (5,541–7,661)	1·860±0·204

of peak effect, M320 was approximately seventy-five times more potent than morphine. The slopes of the regression lines relating log dose of each drug to its effect 30 min after injection were significantly different ($P<0.05$). The slopes at 1 and 3 hr after an injection did not differ significantly from parallelism.

The analgesic potencies of M320 and morphine, determined at various times after parenteral administration to rats, are shown in Fig. 1. The peak effect of morphine, indicated by the lowest ED50 value obtained, occurred 15 to 30 min after either intravenous or subcutaneous administration of the drug, whereas the action of M320 was again slow to develop, peak analgesia occurring approximately 3 hr after administration by either route. Comparison of the ED50s found at the time of peak effect for each of the two drugs indicates that M320 is approximately 400-times more potent than

morphine in this test situation. The results summarized in Fig. 1 suggest that analgesia after M320 administration is much longer lasting than that after equianalgesic doses of morphine. This conclusion was supported by the finding that the time required for the effect of the subcutaneous ED80 to fall to a level equivalent to the ED20 was 38 min for morphine and 854 min for M320.

Tolerance to the analgesic action developed rapidly in rats during administration of daily single doses of M320. Fig. 2 indicates the percentage showing analgesia each

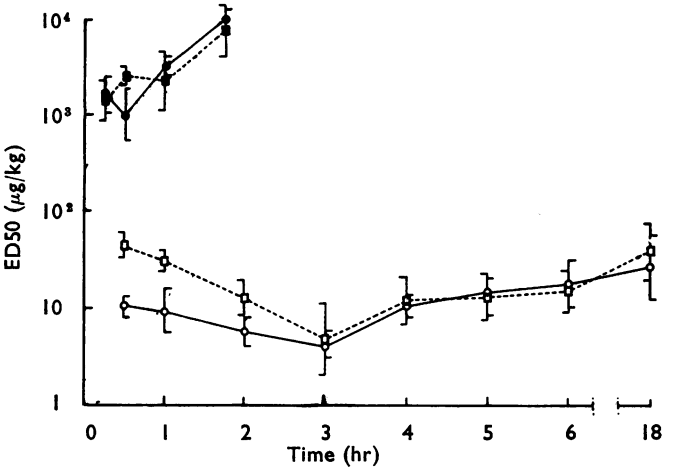


Fig. 1. Analgesic ED50s for M320 hydrochloride and morphine sulphate determined at various times after parenteral administration to rats. Morphine: intravenously ●, subcutaneously ■; M320: intravenously O, subcutaneously □. 95% fiducial limits are indicated by the vertical bars.

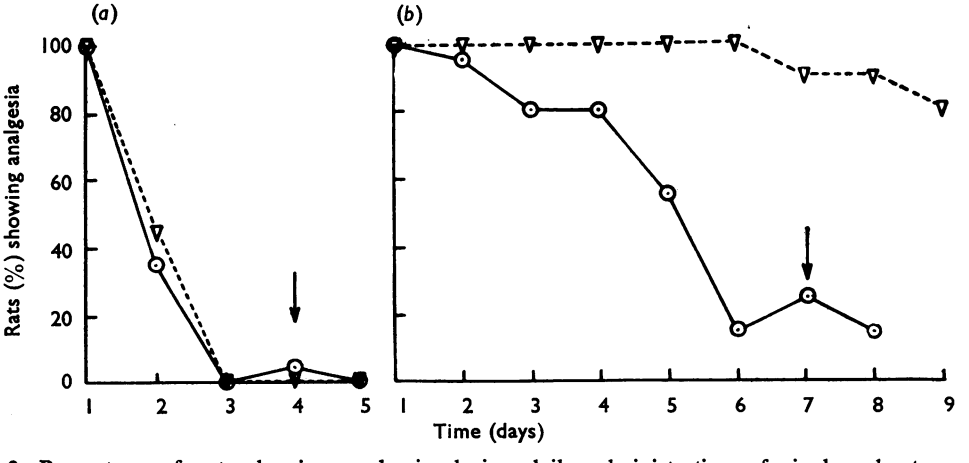


Fig. 2. Percentage of rats showing analgesia during daily administration of single subcutaneous doses of either M320 hydrochloride or morphine sulphate. (*a*) M320: O——O, 132 μg/kg; ▽ - - - - ▽, 440 μg/kg. At the arrow morphine (6.3 mg/kg) was administered instead of M320. (*b*) Morphine: O——O, 6.3 mg/kg; ▽ - · - · - ▽, 21 mg/kg. At the arrow M320 (132 μg/kg) was administered instead of morphine.

day 30 min after the administration of either three or ten times the analgesic ED50s of M320 and morphine. After three daily injections of M320 no animals in either dose group showed analgesia whereas tolerance to doses of morphine, initially causing equivalent effects, developed more slowly. At this time the difference between the drugs was very highly significant ($P<0.001$). Fig. 2 indicates also that cross tolerance to the two drugs occurred; animals that were tolerant to the action of M320 did not show analgesia when injected with morphine and *vice versa*.

Analgesia, catatonia and respiratory depression. The relative abilities of M320 and morphine to cause analgesia, catatonia, and respiratory depression in the rat and guinea-pig are compared in Table 2. In each test using the rat, the slopes of the regression lines, relating log dose of each drug to its effect, did not differ significantly from parallelism ($P>0.05$), and the potency ratio was approximately constant. However, with the guinea-pig, although M320 appeared more potent than morphine in each test situation examined, the regression lines were not parallel, the difference in each case being highly significant ($P<0.01$). For this reason calculation of potency ratios for this species was not attempted. M320 also differed from morphine in the guinea-pig by causing analgesia only at dose levels much higher than those necessary to cause catatonia.

TABLE 2

RELATIONSHIP BETWEEN THE SUBCUTANEOUS DOSE LEVELS OF M320 AND MORPHINE REQUIRED TO CAUSE ANALGESIA, CATATONIA, RESPIRATORY DEPRESSION AND DECREASED GASTROINTESTINAL (GI) PROPULSION 30 MIN AFTER ADMINISTRATION TO RATS AND GUINEA-PIGS

95% Confidence limits are in parentheses, and slopes include the ± standard errors. * Significant differences between the slopes of the regression lines precluded calculation of potency ratios. †ED40

		Morphine sulphate		M320 hydrochloride		
Species	Test	ED50 (mg/kg)	Slope	ED50 (μg/kg)	Slope	Potency ratio
Rat	Analgesia	2·44 (1·97–3·03)	4·330±1·680	45·19 (32·86–62·14)	3·174±1·058	54
	Catatonia	4·88 (3·32–7·16)	2·247±0·912	83·83 (37·12–189·32)	1·006±0·045	58
	Respiratory depression	43·50 (29·92–63·24)	0·679±0·082	931·27 (678·7–1,277·8)	0·514±0·045	47
	Decreased GI propulsion	1·08 (0·91–1·30)	−1·130±0·095	19·15 (15·37–23·85)	−1·108±0·111	56
Guinea-pig*	Analgesia	10·80 (9·62–12·14)	2·398±0·279	10,367 (2,371–45,334)	0·281±0·051	—
	Catatonia	15·99 (12·10–21·13)	1·021±0·186	2·35 (1·97–2·79)	1·680±0·166	—
	Respiratory depression†	222·87 (116·02–428·11)	0·462±0·088	20·58 (11·20–37·85)	0·262±0·035	—
	Decreased GI propulsion	0·018 (0·013–0·025)	−0·514±0·043	0·0031 (0·0024–0·0041)	−0·663±0·047	—

Behavioural changes. The effect of M320 and morphine on co-ordinated locomotor activity of mice is shown in Fig. 3. Activity was reduced by M320 (30 to 100 μg/kg) given subcutaneously. By contrast morphine (10 to 30 mg/kg, subcutaneously) caused an increase in activity which was inhibited by M320 in doses causing depression (Fig. 3,*b*). Elevated (Straub) tails were observed after low doses of M320 (1 to 10 μg/kg) but the effect was not as prominent as that seen after morphine (1 to 10 mg/kg) and an increase in the dose of M320 to levels which decreased locomotor activity led to its

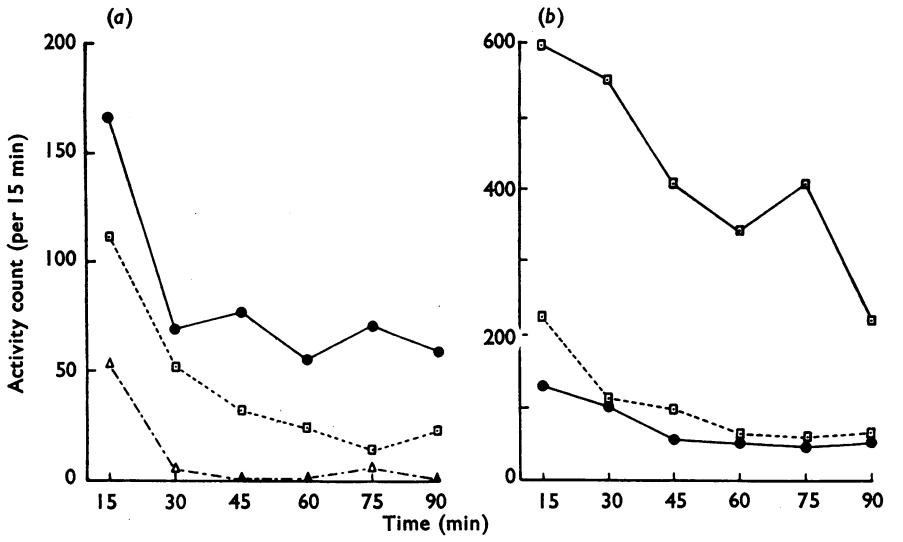


Fig. 3. Co-ordinated locomotor activity of mice. Each point is the mean count recorded using three groups of three mice. (*a*) ●——●, Controls, saline; □- - - -□, M320 30 μg/kg; △- - - -△, M320 100 μg/kg. (*b*) ●——●, Controls, saline; □——□, morphine 30 mg/kg; □- - - -□, morphine 30 mg/kg together with M320 10 μg/kg. All injections were subcutaneous.

abolition. Pinna and corneal reflexes were lost at dose levels of M320 higher than 100 μg/kg and the righting reflex was abolished after 1 mg/kg.

Administration of M320 (44 μg/kg) subcutaneously to rats was followed 3 hr later by rhinorrhea, chromodacryorrhea and abolition of pinna and corneal reflexes, together with marked sedation. The rhinorrhea and chromodacryorrhea were relatively transient lasting for 1 hr whereas the block of reflexes and depression persisted for over 8 hr.

In groups of three or four cats M320, given subcutaneously in low doses, caused hypokinesis, whilst administration of high doses caused excitement followed by sedation. Thus doses of 10 to 30 μg/kg caused sedation, loss of placing reactions and profuse salivation accompanied by mydriasis and submaximal relaxation of the nictitating membranes for over 24 hr; 0.1 to 2 mg/kg caused emesis, followed by excitement for 24 hr which was less marked than that seen after morphine. The excitatory phase was succeeded by one of depression, persisting for several days.

Dogs responded to the intravenous administration of 1 to 10 μg/kg of M320 by a slowly developing narcosis, which took 10 to 60 min to develop and continued for 6 to 24 hr. Intravenous administration of morphine (1 to 10 mg/kg) caused initial excitement for 1 to 3 min followed by sedation. Administration of 5, 10 or 20 μg/kg of M320 to groups of four dogs led to a dose-dependant increase in pain threshold persisting for over 24 hr. These effects occurred most rapidly after injection of the higher doses but at all dose levels developed more slowly than those seen after comparable doses of morphine (5 to 20 mg/kg, intravenously). These doses of M320 caused miosis, salivation, tremors, bradycardia and respiratory depression together with ataxia attributable to hind-limb weakness, whilst administration of larger doses (0.03 to 1 mg/kg) was followed

by complete prostration, miosis, salivation, relaxation of the nictitating membranes, bradycardia, periodic respiration and hypothermia lasting for 18 to 48 hr. The corneal reflex persisted except after very high doses (0.1 to 1 mg/kg). 1 mg/kg was followed 24 hr later by haematuria, lasting several hours in two dogs. The overt depressant effects appeared to occur after doses of M320 much closer to those causing analgesia than was found with morphine, although the number of dogs used at each dose level was insufficient to establish this difference as statistically significant. Emesis followed subcutaneous administration of either 3 μg/kg of M320 or 3 mg/kg of morphine to six dogs. No differences in incidence or extent of vomiting were found between the two drugs but the time elapsing between drug administration and onset of emesis was longer after M320 (mean, 208 min) than after morphine (mean, 4.5 min), the difference being very highly significant ($P \leqslant 0.001$).

In rhesus monkeys 10 μg/kg of M320, subcutaneously, caused profound sedation together with respiratory depression and ptosis lasting for 12 to 24 hr. 100 μg/kg of M320, given by the same route to others, caused narcosis to the point where the animals were completely unresponsive to external stimuli for over 48 hr. 0.5 to 1 mg/kg caused narcosis for several days but with eventual recovery. Haematuria was observed 24 hr after administering 1 mg/kg of M320 subcutaneously to two monkeys.

Gastrointestinal tract

Decreased gastrointestinal propulsion. Gastrointestinal propulsion in rats and guinea-pigs was reduced by M320 at dose levels much lower than those of morphine necessary to cause comparable effects (Table 2). For the two drugs, the regression lines relating dose to effect in the rat did not deviate significantly from parallelism but in the guinea-pig a significant difference in slope was found ($P<0.05$).

Peristaltic reflex. Contractions of the longitudinal muscle and changes in intestinal volume, caused by raising the intraluminal pressure of the guinea-pig isolated ileum, were slowly reduced in the presence of M320. Responses to low pressures were

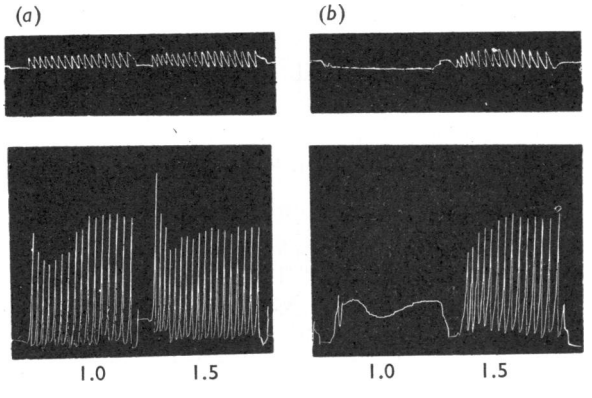


Fig. 4. The effects of M320 on the peristaltic reflex in the guinea-pig isolated ileum. Upper tracing: emptying of the lumen. Lower tracing: contractions of the longitudinal muscle. (*a*) Control; (*b*) 9 min after the addition of M320 to give a bath concentration of 0.1 ng/ml. The figures below the tracings indicate the intraluminal pressure above threshold in cm of water.

preferentially suppressed by bath concentrations of 0.01 to 0.1 ng/ml., those to higher pressures being depressed to a smaller extent (Fig. 4). Abolition of responses to high pressures occurred in the presence of 1 to 10 ng/ml. of M320. Morphine (10 to 100 ng/ml.) caused similar inhibitory effects, confirming the findings of Gyang, Kosterlitz & Lees (1964), but its action developed more rapidly. Nalorphine (10 ng/ml.), or removal of the drug from the bath by washing the preparation, did not modify the blockade caused by M320 but readily reversed the effect of morphine.

Urinary system

The effect of M320 and morphine on the urine output of rats is shown in Fig. 5. Subcutaneous doses of 1 to 3 μg/kg of M320 caused diuresis accompanied by little or no change in sodium excretion. These doses tended to increase potassium excretion but in no experiment were the results significantly different from the controls. By contrast morphine (3 to 30 mg/kg, subcutaneously) exerted an antidiuretic effect. Haematuria occurred 24 hr after subcutaneous doses of 0.1 to 1 mg/kg of M320. Histological examination of the urogenital tract revealed marked epithelial damage in the bladder but no detectable changes in the kidneys or ureters (R. L. F. Dawes, personal communication).

Acute toxicity

The acute intravenous toxicities of M320 and morphine in mice and rats are shown in Table 3. The slopes of the regression lines relating the dose administered in each

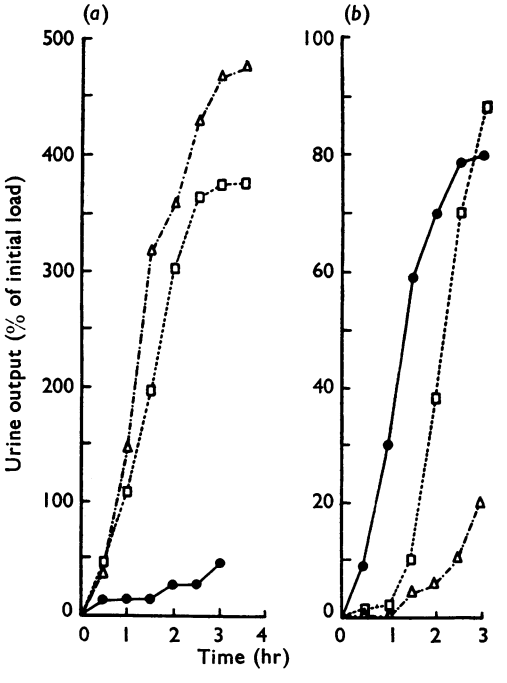


Fig. 5. Urine output of rats receiving either M320 or morphine subcutaneously. (*a*) Saline loaded: M320, □----□ 1 μg/kg, △----△ 3 μg/kg; ●——● controls. (*b*) Water loaded: morphine, □----□ 10 mg/kg, △----△ 30 mg/kg; ●——● controls.

group to the mortality in mice did not differ significantly from parallelism, but in rats the slope obtained with M320 was considerably less than that for morphine. The subcutaneous LD50 in the mouse for either drug was more than 200 mg/kg. In rats the subcutaneous LD50 for M320 was approximately 37 mg/kg and for morphine 185 mg/kg; the regression lines again differed in slope, being 0.1 and 4.7 respectively.

TABLE 3

ACUTE INTRAVENOUS TOXICITIES OF M320 HYDROCHLORIDE AND MORPHINE SULPHATE IN MICE AND RATS

95% Confidence limits are in parentheses

Compound	Species	LD50 (mg/kg)	Slope
M320 hydrochloride	Mouse	36·5 (33·2– 40·2)	8·6
Morphine sulphate	Mouse	223·8 (195·5–256·2)	8·3
M320 hydrochloride	Rat	4·6 (1·45– 14·6)	0·26
Morphine sulphate	Rat	100·0 (63·7–156·9)	3·3

Cardiovascular and respiratory systems

A slowly developing mean fall of 38 mm Hg in systolic blood pressure, lasting for over an hour, followed administration of 10 μg/kg of M320 to eight anaesthetized dogs. Bradycardia developed concomitantly with the fall in arterial blood pressure in four of these animals whose heart rate was recorded. Usually the lowering of systolic blood pressure was accompanied by reduction in pulse pressure and reduction of the reflex pressor responses to bilateral occlusion of the common carotid arteries (Fig. 6). Vagotomy, carried out in two further dogs before administration of the drug, failed to modify the slowing of the heart rate caused by M320. Administration of larger doses of M320 (30 μg/kg) did not depress the systolic pressure further in five of the dogs but caused an additional mean fall of 21 mm Hg in the remainder. The pressor effects of adrenaline, noradrenaline and the ganglion stimulant dimethylphenylpiperazinium and the depressor effects of histamine and acetylcholine were not changed significantly after these doses of M320.

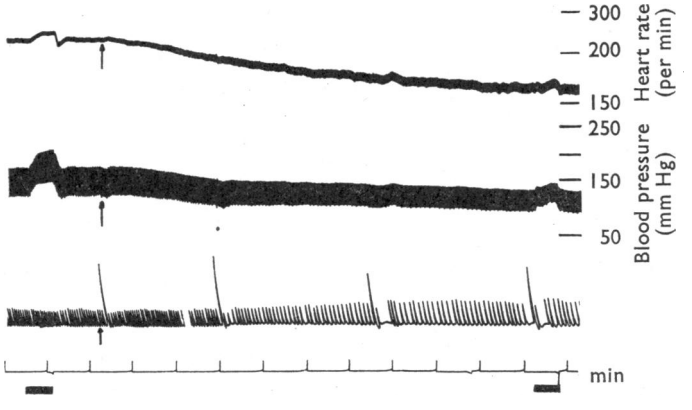


Fig. 6. Physiograph recording from a dog anaesthetized with pentobarbitone. Upper tracing: heart rate; middle tracing: right carotid arterial blood pressure; lower tracing: respiratory movements. At the arrow 10 μg/kg of M320 was injected intravenously. The horizontal bar indicates the period of time the left carotid artery was occluded.

Respiratory depression was caused by intravenous injection of 10 μg/kg of M320, indicated by a gradual decrease in rate and increase in amplitude of respiratory movements. Complete respiratory failure occurred in two of four dogs which had received 100 μg/kg of M320.

Similar cardiovascular and respiratory effects to those described above for the dog were observed in two anaesthetized cats and three anaesthetized monkeys. Both species responded to 30 μg/kg of M320 intravenously with a fall in arterial blood pressure, together with bradycardia and respiratory depression. Respiratory failure occurred in both cats after a total dose of 30 to 100 μg/kg, but respiratory movements persisted in the monkeys even after very large doses (1 mg/kg) of M320.

Antagonism of M320

Analgesia. Antagonism of the analgesic action of M320 in the rat by nalorphine, a specific antagonist of potent analgesics, occurred most readily during the early stages of analgesia. When analgesia had fully developed, relatively large doses of nalorphine failed to antagonize it. In Fig. 7,*b* the percentage of rats showing analgesia is plotted against the dose of M320 administered subcutaneously 30 min earlier. Nalorphine, given by the same route and at the same time, caused the regression line relating the dose of M320 to the analgesic effect to move to the right, the extent of the shift depending on the dose of antagonist. Deviations from parallelism were not significant. It was calculated that after 10 mg/kg of nalorphine approximately 100-times the dose of M320 had to be given to obtain the same effect as that seen when no antagonist had been administered. Testing 3 hr after subcutaneous administration of M320 had the effect of moving the regression line to the left (Fig. 7,*a*), in keeping with the slow onset of the drug's action, and at this time the number of rats showing analgesia at each dose level was not altered significantly by the administration of 10 mg/kg of nalorphine 30 min earlier.

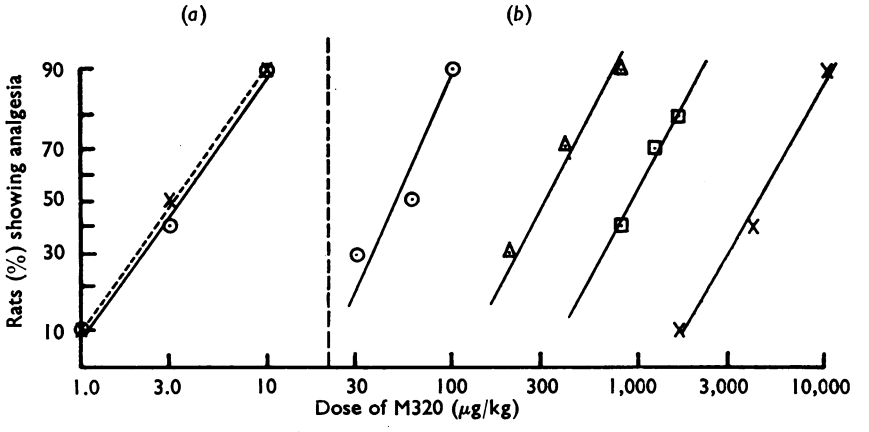


Fig. 7. Effect of nalorphine on analgesia in rats caused by M320, both drugs given subcutaneously. The graph shows the percentage of rats showing analgesia plotted against the log dose of M320. (*a*) M320 injected 3 hr before testing; O——O, M320 alone; ×----×, M320 followed by the administration of 10 mg/kg of nalorphine, 30 min before testing. (*b*) M320 injected 30 min before testing: O——O, M320 alone; △——△, M320 with 2.5 mg/kg of nalorphine; □——□, M320 with 5 mg/kg of nalorphine; ×——×, M320 with 10 mg/kg of nalorphine.

Respiratory depression. The decrease in respiratory minute volume and other overt depressant effects caused by intravenous administration of 10 μg/kg of M320 to four dogs were only slightly reversed by nalorphine in doses up to 25 mg/kg. Levallorphan (up to 15 mg/kg) either did not antagonize or further increased the respiratory depression in four other dogs. However, decreased respiratory minute volume and prostration caused by large doses of morphine (10 to 20 mg/kg) were antagonized completely by either nalorphine (1 to 10 mg/kg) or levallorphan (0.1 to 3 mg/kg).

Bemegride (1 to 5 mg/kg) reversed for 5 to 10 min the decreased respiratory minute volume caused by M320 (10 to 50 μg/kg) in two dogs. Amiphenazole (1 to 10 mg/kg) transiently antagonized both respiratory and other depressant effects of these doses of M320 in five others, the animals appearing to behave normally for approximately 20 min before resuming a state of deep narcosis.

All the drugs referred to in this section were given intravenously.

DISCUSSION

These results show that M320 possesses powerful and long lasting central depressant properties. In several test situations the drug was found to be several hundred times more potent than morphine and to have a duration of action approximately twenty times as long.

Although M320 and morphine have many properties in common, interesting differences were also found. Whereas in the rat no difference could be detected between the drugs in their relative abilities to cause analgesia, catatonia, decreased gastrointestinal propulsion and respiratory depression, the diuretic action of M320 contrasted sharply with the anti-diuresis seen after morphine. Furthermore, dissimilarities were also found between the actions of the two drugs in the guinea-pig, and of particular interest was the observation that M320, unlike morphine, exerted a catatonic effect at much lower doses than those required to cause analgesia.

Nalorphine and levallorphan did not readily antagonize the depressant actions of M320 either *in vitro* or *in vivo*. Nalorphine failed to reverse the block of the peristaltic reflex in the isolated ileum of the guinea-pig, and both morphine antagonists caused little antagonism of M320's respiratory depressant and overt narcotic effects in the dog. The more detailed studies, measuring analgesia in the rat, indicated that nalorphine will competitively antagonize M320 provided it is administered before the time of maximum effect. It appears, therefore, that after its depressant action has developed fully M320 has a greater affinity for the receptor sites involved than has morphine, and this may partly explain the much longer duration of its action.

Narcotic analgesics cause a combination of central depression and stimulation and it is well known that the relative prominence of these effects varies with different drugs and in different species. Following M320 depressant actions predominated almost exclusively, stimulatory effects being much less marked in species in which central excitation predominates after morphine. Thus, although mice responded to low doses of M320 with the Straub-Hermann reaction, maximal tail elevation was never as great as that seen after morphine. Moreover, in a contrast to morphine, increased doses of M320 led to sedation, indicated by a decrease in co-ordinated locomotor activity and abolition

of the Straub effect. The lack of excitatory effects caused by M320 was further emphasized by the finding that sedative doses of the drug inhibited the increase in locomotor activity and tail elevation caused by morphine. Similarly in dogs, whereas intravenous morphine caused a brief preliminary period of excitement succeeded by sedation, administration of M320 by the same route was followed only by a slowly developing narcosis. Overt excitement was seen only in cats after large doses of M320.

Many of the actions of M320 were slower to develop than those of morphine. *In vitro,* inhibition of the peristaltic reflex in the guinea-pig ileum developed more slowly in the presence of M320 than in the presence of morphine. *In vivo,* maximum analgesia in mice and rats and emesis in dogs followed much later after subcutaneous doses of M320 than after doses of morphine given by the same route. Similar differences in the rate of onset of analgesia were found after intravenous administration of the two drugs to rats and dogs.

Tolerance to the analgesic action of M320 occurred during the administration of daily single doses of the drug to rats, and developed more rapidly than that seen during daily administration of equivalent doses of morphine. Faster onset of tolerance to the longer acting M320 is to be expected as frequency of administration, and therefore presumably duration of narcosis, has been shown to influence the rate of onset of tolerance to morphine (Wikler, 1950).

The cardiovascular actions of M320 resembled those of morphine. A slowly developing but long lasting hypotension accompanied by bradycardia occurred in anaesthetized cats, dogs and monkeys following M320 intravenously. Blockade of baroreceptor reflexes may be partly responsible for these effects as reflex responses to occlusion of the carotid artery were usually depressed concomitantly.

The acute toxicity of M320 in the mouse relative to that of morphine appears to be low. In the rat accurate comparison of the toxicities of the two drugs at a single dose level could not be made because of the marked difference in the slopes of the regression lines relating dose to mortality. That M320 tends to be relatively more toxic than morphine at low parenteral doses in this species is indicated by the much smaller slopes of its regression lines; the much longer duration of action of the former drug would contribute to its toxicity. Respiratory depression was caused by M320 in all species examined and usually this was the cause of death. Haematuria occurred after administration of large doses of M320 to rats, dogs and monkeys and, in the rat, was due to cystitis. This effect, in at least the latter species, may be attributed to trauma resulting from increased intravesical pressure. M320 probably causes spasm of the vesical sphincter analogous to that seen after morphine and this, together with its powerful diuretic action, would result in a massive and long lasting increase of pressure within the urinary bladder. Support for this hypothesis comes from the observation that haematuria occurred only after a considerable time had elapsed from the moment of injection and also from the finding of Mehrotra (1953) that ligation of the urethra of rats is followed within 6 to 24 hr by the occurrence of inflammatory changes in the bladder wall.

The close similarity between the chemical structures of M320 and morphine suggests that many of the morphine-like effects seen after administration of the former drug can be ascribed to actions at similar receptor sites. The dissimilarities between the effects

of the two drugs can perhaps be related to physicochemical differences. Both quantitative and qualitative differences can be attributed to the much higher lipid solubility of M320, as at *p*H 7.3 its carbon tetrachloride: water partition coefficient is greater than 2,000 whereas that of morphine is less than 0.001 (Daglish & McDougall, personal communication). Its lipophilic character may allow M320 some degree of selective uptake into the central nervous system, contributing towards the increase in its potency and changing its central spatial distribution relative to morphine with consequent alteration in the spectrum of its pharmacological effects.

SUMMARY

1. The acute effects of M320 (Reckitt) [*N*-(cyclopropylmethyl)-19-isopentylnororvinol hydrochloride], a powerful morphine-like central depressant, have been investigated and compared with those of morphine.

2. M320 resembled morphine by causing analgesia, catatonia, respiratory depression and inhibition of gastrointestinal propulsion.

3. M320 was 50- to 500-times more potent than morphine as an analgesic in the mouse, rat and dog; its effects developed more slowly and persisted approximately twenty times as long.

4. Although in the rat it was not possible to detect any dissociation of several morphine-like effects, M320 contrasted with morphine by causing diuresis. In the guinea-pig, M320 differed from morphine by exerting an analgesic action at doses much higher than those causing catatonia.

5. The morphine antagonists, nalorphine and levallorphan, did not reverse the decrease in respiratory minute volume and narcosis caused by M320 in the dog. However, nalorphine was a competitive antagonist of M320 in the rat provided it was administered before the time of the maximum effect of the latter drug.

6. Tolerance to the analgesic action occurred in rats during the administration of daily single doses of M320, and developed more rapidly than that seen after daily doses of morphine.

7. Central excitatory effects after M320 were much less prominent than those of morphine in mice and cats.

8. The acute toxicity in the mouse of M320 was low, but small doses of the drug were relatively more toxic than those of morphine in the rat. Haematuria followed administration of large doses of M320 to rats, dogs and monkeys.

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