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Interactions of narcotic antagonists and antagonist-analgesics

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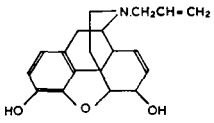
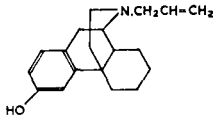
In the rat the potent narcotic antagonist *N*-cyclopropylmethyl-6,14-*endo*ethano-7α-(1-hydroxy-1-methylethyl)-tetrahydronororipavine (M5050, Reckitt), which itself lacks analgesic activity, resembled naloxone in its capacity to reverse the anti-nociceptive effects of morphine antagonist-analgesics. The nociceptive stimulus employed was bradykinin administered by an intra-arterial route. ED50 values were established for the reversal by M5050 of the analgesic effect of nalorphine, levallorphan, pentazocine and some newer compounds. When antagonist-analgesics were given concomitantly with morphine the response varied from antagonism of the analgesic effect of the morphine to synergism, depending on the dose combination.

THE narcotic antagonist naloxone differs from well-established morphine antagonists, such as nalorphine and levallorphan, in possessing no antinociceptive properties of its own in animal tests (Blumberg, Wolf & Dayton, 1965). Again, unlike nalorphine, naloxone does not produce dose-related analgesic effects in man, nor is its use associated with respiratory depression or psychotomimetic side-effects (Foldes, 1964; Lasagna, 1965; Sadove, Balagot & others (1963). Recently, Blumberg, Dayton & Wolf (1966) observed that naloxone also antagonizes the analgesic effects of nalorphine, pentazocine, cyclazocine, cyclorphan and levallorphan in mice and rats using intraperitoneal phenyl-*p*-benzoquinone as the nociceptive agent.

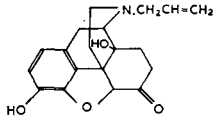
One compound from an extensive series of bridged-ring derivatives of tetrahydrothebaine (Bentley, 1967) showed a preliminary pharmacological profile sufficiently similar to naloxone to stimulate our making a comparison. The lack of antinociceptive activity of *N*-cyclopropylmethyl-6,14-*endo*ethano-7α-(1-hydroxy-1-methylethyl)-tetrahydronororipavine (M5050, Reckitt) in the rat tail-pressure, the mouse phenyl-*p*-benzoquinone and the rat bradykinin tests has already been reported (Blane, 1967).



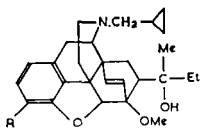
Nalorphine



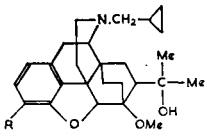
Levallorphan



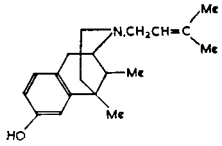
Naloxane



M 5217 R=OH
M 5205 R=OMe



M 5050 R=OH
M 5046 R=OMe



Pentazocine

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We have now used the bradykinin test in the rat, first to compare M5050 with naloxone, and subsequently to examine the interaction of M5050 with antagonist-analgesics, some of which themselves are new (see formulae).

The interaction of the antagonist-analgesics nalorphine and pentazocine with morphine has also been examined.

Experimental

Antinociception in the rat. Male SPF-derived Sprague–Dawley rats of between 250 and 350 g were used in groups of 5 or 10. The minimum dose of intra-arterial bradykinin required to consistently produce dextro-rotation of the head and flexion of the right fore-limb was established for each rat and subsequently repeated at regular intervals to provide the basis for a time-response curve. Disappearance of this syndrome after drug administration was taken as the criterion for scoring an analgesic effect. Details of this procedure were given by Blane (1967).

Antinociception in the mouse. Female mice (C.D. strain) were used in groups of 5 or 10. Antinociceptive activity was determined as the dose of drug required to reduce by 50% the number of abdominal stretches (writhes) caused by intraperitoneal injection of 2 mg/kg of phenyl-*p*-benzoquinone (Hendershot & Forsaith, 1959).

Narcotic antagonist activity. Antagonist activity was assessed as that dose of drug in the rat tail-pressure test, which reduced to 50% the total analgesia caused by 10 mg/kg morphine sulphate administered subcutaneously (Green, Ruffell & Walton, 1954).

Interaction of antagonists with antagonist-analgesics. The bradykinin test was used. Doses were based on preliminary findings. When antagonists of the naloxone type were evaluated against antagonist-analgesics all animals initially received a dose of the antagonist-analgesic which was nearest to that blocking the bradykinin-induced syndrome in 9 out of 10 animals (ED 86–94). Groups of animals also received at the same time one of a series of doses of the antagonist under test. The results were expressed in terms of the dose of antagonist required to effect a 50% reduction in the number of animals showing analgesia at the time of the peak effect, i.e. from about 86–94% to about 43–47%.

Interaction of morphine with nalorphine and pentazocine. The response to bradykinin of rats given an analgesic ED20 of morphine followed immediately by one of a graded series of doses of nalorphine was assessed. So too was the response of animals given an analgesic ED20 of nalorphine and different doses of morphine. Similar experiments were made using morphine and pentazocine.

Statistical examination of data. Agonist ED50 values and their 95% confidence limits were estimated using the method of Litchfield & Wilcoxon (1949). ED50 values in the interaction studies were estimated (Litchfield & Wilcoxon) from the regression lines relating reduction in the number of animals showing analgesia to the logarithm of the dose of antagonist. ED50 values were always assessed at the time of peak drug or drug mixture effect.

Drugs. Morphine, levallorphan, pentazocine and the Reckitt compounds: M5046 [*N*-cyclopropylmethyl-6,14-*endo*ethano-7α-(1-hydroxy-1-methylethyl)-tetrahydronorthebaine], M5050 [*N*-cyclopropylmethyl-6,14-*endo*ethano-7α-(1-hydroxy-1-methylethyl)-tetrahydronororipavine], M5205 [*N*-cyclopropylmethyl-6,14-*endo*etheno-7α(1-(*R*)-hydroxy-1-methylpropyl)-tetrahydronorthebaine] and M5217[*N*-cyclopropylmethyl-6,14-*endo*etheno-7α-(1-(*R*)-hydroxy-1-methylpropyl)-tetrahydronororipavine], the chemistry of which have been described by Bentley (1967).

All drugs, other than bradykinin, were administered subcutaneously. Where the interaction of two drugs was studied in a single animal, both drugs were injected simultaneously into different sites. All doses are expressed as the weight of the salt. Morphine was used as the sulphate, nalorphine as the hydrobromide, levallorphan as the tartrate. The other compounds were hydrochlorides. Bradykinin was given as a solution of the pure synthetic peptide (Sandoz) in 0·9% saline.

Results

Reversal by naloxone and M5050 of the antinociceptive effects of antagonist-analgesics. Naloxone, like M5050, had no activity at doses up to 100 mg/kg in antinociception tests. Each drug was then administered in

TABLE 1. ANTAGONISM OF NALORPHINE ANALGESIA BY NALOXONE AND M5050 IN THE BRADYKININ TEST. The dose of nalorphine is, to the nearest mg, that which when given alone blocks the response to bradykinin in 9 out of 10 animals (ED87). All drugs given subcutaneously.

	Naloxone			M5050		
Nalorphine mg/kg	Drug mg/kg	Block of nociception %	ED50 mg/kg (95% limits)	Drug mg/kg	Block of nociception %	ED50 mg/kg (95% limits)
10	1·0	70 (7/10)	4·1 (2·1–7·6)	0·005	80 (8/10)	0·016 (0·010–0·024)
10	2·5	60 (6/10)		0·010	60 (6/10)	
10	5·0	40 (4/10)		0·020	40 (4/10)	
10	10·0	20 (2/10)		0·030	20 (2/10)	

TABLE 2. INTERACTION OF M5050 WITH ANTAGONIST-ANALGESICS. Drugs are ranked in descending order of morphine antagonist activity.

Antagonist-analgesic	Analgesic activity, s.c. ED50 (mg/kg)		Morphine antagonist activity in rats ED50 (mg/kg)	Reversal of anti-bradykinin action in rats by M5050 ED50 (mg/kg) 95% confidence limits in parentheses
	Anti-writhing (mice)	Anti-bradykinin (rats)		
M5217	0·029	0·39	0·021	0·135 (0·090–0·198)
Levallorphan	2·4	125·0	0·30	0·120 (0·053–0·270)
Nalorphine	2·1	4·0	0·48	0·016 (0·010–0·026)
M5046	1·5	2·4	5·4	0·008 (0·003–0·015)
Pentazocine	3·0	1·85	≃30	0·013 (0·009–0·018)
M5205	2·5	2·5	44	0·008 (0·004–0·018)

a graded series of doses to rats at the same time as sufficient nalorphine to abolish the response to intra-arterial bradykinin in 9 out of 10 animals. Nalorphine analgesia was antagonized by M5050 and naloxone in a dose-related manner (Table 1); M5050 was more than 250 times the more potent. M5050, in a graded series of doses, was then evaluated in rats given doses close to the ED90 of antagonist-analgesic compounds including levallorphan and pentazocine, as well as M5046, M5205 and M5217.

Table 2 shows the antinociceptive and morphine-antagonist actions of these compounds, as well as the ED50 values for reversal of analgesia by M5050 in the bradykinin test. Values for antagonism of analgesia caused by pentazocine, M5046 and M5205 were similar to that already established against nalorphine analgesia, and ranged between 0·008 and 0·013 mg/kg. However, we found levallorphan and M5217 analgesia to be less readily antagonized by M5050, the ED50 values for reversal of analgesia being 0·120 mg/kg and 0·135 mg/kg respectively. Blumberg & others (1966) also reported a high ED50 for reversal of levallorphan analgesia, using naloxone.

TABLE 3. EFFECT OF MIXTURES OF MORPHINE AND NALORPHINE ON BRADYKININ-INDUCED NOCICEPTION IN THE RAT. Drugs given synchronously subcutaneously. Analgesic values represent means for block of nociception in groups of at least 10 animals.

Experimental design	Morphine (A)		Nalorphine (B)		A + B Observed/ expected analgesia*	Comment
	Dose (mg/kg)	Expected analgesia %	Dose (mg/kg)	Expected analgesia %	%	
Nalorphine dose fixed, morphine dose varied	0·1	<1	3·0	20	30/<21	Synergism
	0·2	3	3·0	20	60/23	Synergism
	0·4	10	3·0	20	80/30	Synergism
	0·6	20	3·0	20	80/40	Synergism
Morphine dose fixed, nalorphine dose varied	0·6	20	0·1	0	0/20	Antagonism
	0·6	20	0·5	<1	20/<21	Addition
	0·6	20	1·0	<1	30/20	Synergism?
	0·6	20	2·0	7	40/27	Synergism
	0·6	20	3·0	20	80/40	Synergism

* Analgesia expected on the basis of a simple additive effect of the two drugs.

Interaction of antagonist-analgesics with morphine. Mixtures of morphine and nalorphine were given to rats receiving analgesic doses of bradykinin at regular intervals. Table 3 shows that a range of doses of morphine, which would be expected to cause from less than 1% up to 20% analgesia in this test, acted synergistically with a dose of nalorphine (3 mg/kg) which would itself produce analgesia in 20% of animals. When the morphine dose was fixed at the 20% analgesia level (0·6 mg/kg) and the nalorphine dose varied from the zero to 20% effect, the overall effect changed from apparent antagonism with the lowest dose of nalorphine, through simple addition, to synergism with the 3 mg/kg nalorphine dose. However, except at one or two points, the synergism was not highly significantly different from an expected value for simple additive effect ($P \simeq 0{\cdot}05$) in either experiment. Even very large doses of nalorphine did not cause 100% of animals to show analgesia.

Like nalorphine, pentazocine in very low doses (0·35 mg/kg) antagonized the analgesic effect of an ED20 or morphine. Intermediate dose levels

of pentazocine (0·63 and 1·25 mg/kg) acted additively with morphine, but with the 2·5 and 5·0 mg/kg doses of pentazocine there was a return to antagonism (Table 4).

TABLE 4. EFFECT OF MIXTURES OF MORPHINE AND PENTAZOCINE ON BRADYKININ-INDUCED NOCICEPTION IN THE RAT. Drugs given synchronously subcutaneously. Analgesic values represent means for block or nociception in groups of at least 10 animals.

Experimental design	Morphine (A)		Pentazocine (B)		A + B Observed/ Expected analgesia* %	Comment
	Dose (mg/kg)	Expected analgesia %	Dose (mg/kg)	Expected analgesia %		
Morphine dose fixed, pentazocine dose varied	0·6	20	0·35	10	0/30	Antagonism
	0·6	20	0·63	20	40/40	Addition
	0·6	20	1·25	40	60/60	Addition
	0·6	20	2·5	80	40/100	Antagonism
	0·6	20	5·0	60	0/80	Antagonism

* Analgesia expected on the basis of a simple additive effect of the two drugs.

Discussion

Our findings with naloxone and also with M5050 confirm the report of Blumberg & others (1966) that morphine antagonists of this type, which themselves lack analgesic activity, are capable of reversing the antinociceptive effects of antagonist-analgesics, as well as an agonist-analgesic such as morphine. M5050 was exceptionally potent in this respect.

TABLE 5. SUMMARY OF ACTIONS OF ANTAGONIST-ANALGESICS

Antagonist-analgesic	Reversal of anti-bradykinin action in rats by M5050 ED50 (mg/kg)	Ratio: $\frac{\text{Analgesia in bradykinin-rat}}{\text{Morphine antagonism in rat tail}}$
Levallorphan	0·120	416·0
M5217	0·135	18·6
Nalorphine	0·016	8·3
M5046	0·008	0·44
Pentazocine	0·013	0·062
M5205	0·008	0·057

The values for reversal by M5050 of the analgesia caused by antagonist-analgesics in the bradykinin test may be used as a basis to divide these compounds into two categories (Table 5); firstly, compounds having an analgesic action which is reversed by a low dose of M5050 (0·008–0·016 mg/kg), including nalorphine and pentazocine which are known to have an analgesic action in man; secondly, compounds for which the reversal ED50 is approximately 10 times greater, including levallorphan, which appears to lack useful analgesic activity in man (Foldes, 1964; Keats & Telford, 1966, 1967). The ED50 values within these groups are statistically similar but there is a high level of significance ($P < 0·001$) to the difference between any member of one group and any member of the other group. The figures in the second column of Table 5 represent the ratio analgesia (as assessed in the bradykinin test): morphine antagonism; the compounds are ranked in descending order of this ratio.

The compounds for which this ratio is high—levallorphan and M5217—are those which had high values for reversal of analgesia by M5050. By contrast, the analgesia:antagonism ratio is low for the remaining antagonist-analgesics which were reversed by a low dose of M5050.

We interpret this to suggest that levallorphan and M5217 have high receptor affinity with low analgesic efficacy. As such they are intermediate between the other antagonist-analgesics and the antagonists, such as M5050, which have very high receptor affinity with negligible analgesic efficacy.

Low doses of nalorphine and pentazocine antagonized the analgesic effect of morphine in the bradykinin test while with higher doses there was evidence of summation and perhaps synergism between the analgesic actions of the two types of drugs. Houde, Wallenstein & others (1967) report that appropriate morphine-pentazocine combinations caused more analgesia in cancer patients than either drug alone. At least with pentazocine, in our rats still higher doses resulted in fewer, and eventually no animals showing analgesia, which suggests that this drug can antagonize itself as the dose is increased beyond a certain threshold.

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