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Effects of opiates and opiate antagonists on the Straub tail reaction in mice

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1. Subcutaneous injections of opiates produced the Straub tail reaction in mice. The potencies of the opiates in mice were consistent with previous estimates of the analgesic potencies in animals and in man.

2. The potencies of sixteen antagonists in counteracting the reaction were consistent with those previously obtained with the rat tail-flick test.

3. The (−) isomers of four benzomorphan derivatives were much more potent in counteracting the reaction than their (+) isomers and about twice as potent as their racemates. The activity of the isomers seemed to follow Pfeiffer's rule: the lower the effective dose of a drug, the greater the difference in the pharmacological effects of the optical isomers. One of the *trans* isomers acted like an opiate, while its *cis* isomer acted like an antagonist.

4. Naloxone and nalorphine fulfilled conventional criteria for competitive antagonism, whereas atropine and the (−) and the (+) isomers of pentazocine and of cyclazocine did not do so.

5. The Straub tail test seems to be useful for studying structure-activity relations among opiates and opiate antagonists.

Support for the suggestion that certain N-substituted derivatives of opiates antagonize effects of opiates by competing at a common receptor site comes largely from studies in which these antagonists are devoid of opiate-like effects (Martin, 1967). In studying the interaction of several classes of opiates with nalorphine and with levallorphan in the rat tail-flick test, Grumbach & Chernov (1965) noted that the dose of antagonist necessary to reduce the effect of equiactive doses of opiates to a fixed degree was constant and that the ratios of opiate and antagonist doses producing a fixed response were constant for two meperidine congeners over a wide range of doses. Using the number of mice affected with lenticular opacities as an index of the interaction of opiates with nalorphine, Cox & Weinstock (1964) found that the "pA_2" value for each opiate with nalorphine was about the same. Likewise, this held for nalorphine in the tail pressure test in rats (Blane, Boura, Fitzgerald & Lister, 1967).

Using the Straub tail reaction in mice, we attempted to confirm and to extend these results. Structure-activity relations for optical and geometric isomers of benzomorphan derivatives were studied because changes in the isomerism of these

derivatives evidently not only affects the degree of activity exhibited but whether opiate-like or antagonist effects are exhibited (Archer, Harris, Albertson, Tullar & Pierson, 1964; Archer & Harris, 1965; Tullar, Harris, Perry, Pierson, Soria, Wetterau & Albertson, 1967; Pearl, Aceto & Harris, 1968).

Methods

Mice were injected subcutaneously with either an opiate antagonist or distilled water and immediately afterwards with an opiate: twenty-five mice with each dose of opiate and fifteen mice with each dose of opiate antagonist and of opiate. The Straub reaction was defined as elevation of the tail at angles greater than 45°. The number of mice showing the Straub reaction from 15 to 25 min after injection was tabulated. To minimize effects of noise on the reaction, the experiment was conducted in a sound-attenuated room. Doses are reported in mg/kg of the free base. Salts of the drugs appear elsewhere (Pearl *et al.*, 1968).

Cox & Weinstock (1964) described a method for computing "pA_2" values in whole animals in which the concentration of the antagonist at the site of action is unknown. In brief, the negative log of the molar dose of antagonist injected into the animals on a weight basis is taken as the best approximation of the concentration of the antagonist at the site of action.

In the present study at least three doses of each drug were used to estimate the ED50 according to the method of Litchfield & Wilcoxon (1949). Dose ratios (x)

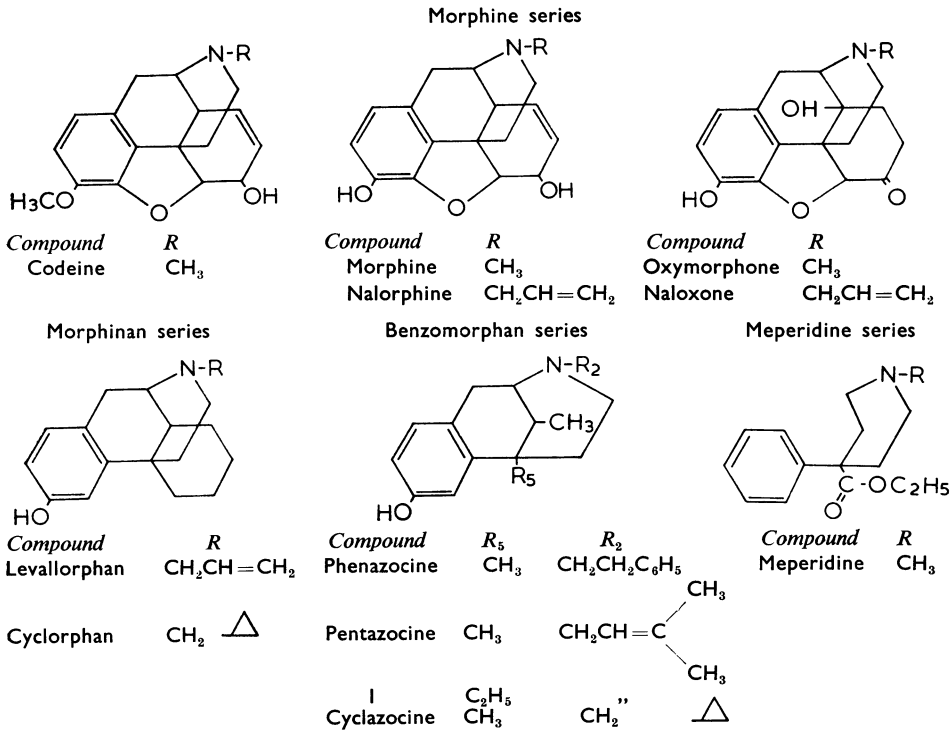

FIG. 1. Structural formulae of opiate derivatives: 5-ring morphine series, 4-ring morphinan series, 3-ring benzomorphan series, and 2-ring meperidine (pethidine) series.

were calculated by dividing the ED50 for the opiate alone by that for the opiate in the presence of the antagonist. Log (x-1) was plotted against N, the negative logarithm of the molar dose (per 100 g body weight) of the antagonist. The "pA_2" values for the antagonists were calculated from the point of intersection of the regression line with the abscissa (Arunlakshana & Schild, 1959; Cox & Weinstock, 1964).

To aid in evaluating the effects of opiates and opiate antagonists on the Straub reaction, the doses producing the reaction were compared with subcutaneous doses necessary to produce other overt effects: hypoactivity in photocell units, inability to remain on a rotating rod, convulsions, and deaths. With the exception of doses for lethality, the doses were taken from one of our previous studies (Pearl *et al.*, 1968). Mr. Leon P. Duprey kindly provided us with 24 hr LD50 values for many of the drugs. These comparisons appear in the results section. Comparisons of our results with those obtained by others with different tests in animals and in man appear in the discussion section.

Drugs

With the exception of atropine sulphate, all the drugs tested were opiate derivatives. Figure 1 shows the structural formulae of the opiate derivatives. Optical and geometric isomers in the benzomorphan series are designated in the results section. Geometric isomerism in this series arises from the disposition of the angular alkyl groups at C-5 and C-9 (Tullar *et al.*, 1967). At C-5 the alkyl group is fixed by the rigid geometry of the fused tricyclic system. At C-9 the *cis* or α isomers have the alkyl group orientated in an axial position away from the nitrogen; the *trans* or β isomers have the alkyl group orientated in an equatorial position toward the nitrogen.

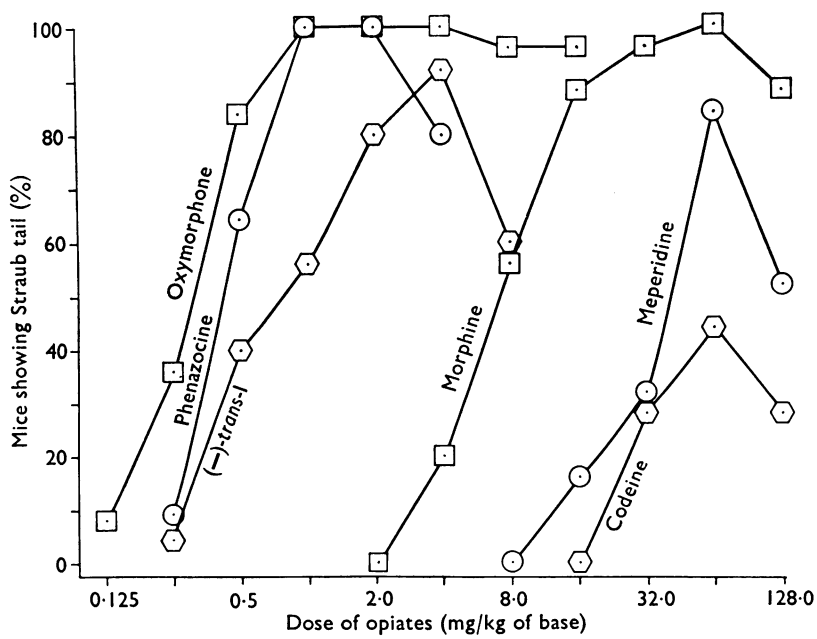

FIG. 2. Percentage of mice showing the Straub reaction after subcutaneous injections of opiates.

TABLE 1. *Potency of opiate antagonists in counteracting the Straub reaction produced by morphine*

Antagonist	ED50 (95% confidence limits) Morphine (16 mg/kg)	Morphine (32 mg/kg)	Slope function: ED50/ED16 Morphine (16 mg/kg)	Morphine (32 mg/kg)
Naloxone	0·014 (0·009–0·022)	0·039 (0·024–0·062)	3·1	2·5
(−)-*cis*-Cyclazocine	0·015 (0·010–0·023)	0·039 (0·026–0·058)	3·0	2·6
Nalorphine	0·23 (0·13–0·40)	0·55 (0·37–0·82)	4·6	2·8
(−)-*cis*-Pentazocine	1·8 (1·4–2·2)	3·0 (2·0–4·4)	2·0	2·1
(+)-*cis*-Cyclazocine	9·3 (5·6–15)	14 (9·0–21)	3·2	2·9
(+)-*cis*-Pentazocine	9·4 (6·3–14)	15 (9·8–23)	2·2	1·8
Atropine	10 (6·2–16)	36 (23–56)	4·0	3·4

Two doses of morphine were tested: 16 mg/kg produced the reaction in 88% of the mice, and 32 mg/kg produced the reaction in 96% (Fig. 2). The antagonist ED50 was defined as the dose of antagonist at which 50% of the mice exhibited the reaction in the presence of the opiates and was estimated according to the method of Litchfield & Wilcoxon (1949). Doses of all drugs are in mg/kg of base.

TABLE 2. *Potency of opiate antagonists in conuteracting the effects of opiates on the Straub reaction*

Antagonist	Isomerism Optical	Isomerism Geometric	ED50 (95% confidence limits) Meperidine (64 mg/kg)	Morphine (16 mg/kg)	Phenazocine (1 mg/kg)	Slope function: ED50/ED16 Meperidine (64 mg/kg)	Morphine (16 mg/kg)	Phenazocine (1 mg/kg)
Naloxone	(−)		0·006 (0·004–0·008)	0·014 (0·009–0·022)	0·021 (0·014–0·029)	2·3	3·1	2·5
Cyclazocine	(−)	*cis*	0·006 (0·004–0·009)	0·015 (0·010–0·023)	0·021 (0·013–0·033)	2·9	3·0	3·6
Cyclazocine	(±)	*cis*	0·015 (0·009–0·024)	0·041 (0·026–0·064)	0·061 (0·039–0·095)	3·0	2·4	1·9
Levallorphan	(−)		0·020 (0·13–0·30)	0·063 (0·045–0·088)	0·058 (0·042–0·080)	2·8	1·6	1·9
Cyclazocine	(−)	*trans*	0·024 (0·016–0·035)	0·025 (0·017–0·038)	0·036 (0·024–0·053)	2·6	2·2	2·6
Cyclorphan	(±)	*cis*	0·024 (0·016–0·035)	0·031 (0·021–0·045)	0·046 (0·031–0·069)	2·1	2·5	2·8
Cyclazocine	(±)	*trans*	0·034 (0·024–0·048)	0·048 (0·035–0·066)	0·054 (0·034–0·086)	1·9	1·8	3·3
Nalorphine	(−)		0·094 (0·062–0·14)	0·23 (0·13–0·40)	0·27 (0·16–0·44)	2·3	4·6	2·0
I	(−)	*cis*	0·63 (0·42–0·94)	1·9 (1·5–4·1)	2·1 (1·4–2·4)	2·2	2·5	3·1
Pentazocine	(−)	*cis*	0·66 (0·40–1·1)	1·8 (1·4–2·2)	1·8 (1·2–2·7)	4·1	2·0	1·8
Pentazocine	(±)	*cis*	1·9 (1·3–2·9)	3·4 (2·2–5·1)	5·0 (3·7–7·0)	2·6	2·3	1·9
I	(±)	*cis*	2·2 (1·6–3·0)	2·1 (1·5–2·9)	4·8 (3·4–6·4)	1·9	2·3	1·8
Cyclazocine	(+)	*cis*	3·0 (1·9–4·8)	9·3 (5·6–15)	13 (8·3–20)	3·6	3·3	2·4
I	(+)	*cis*	7·0 (4·2–12)	8·5 (6·1–12)	14 (9·0–20)	3·5	1·9	2·2
Atropine	(±)		8·8 (4·7–16)	10 (6·2–16)	19 (12–29)	6·7	4·0	2·8
Pentazocine	(+)	*cis*	11 (8·4–15)	9·4 (6·3–14)	16 (12–22)	1·8	2·2	2·1
Cyclazocine	(+)	*trans*	>32	>32	>32	—	—	—

Details as in Table 1. Meperidine (64 mg/kg) produced the Straub reaction in 84% of the mice, morphine (16 mg/kg) produced the reaction in 88% of the mice, and phenazocine (1 mg/kg) produced the reaction in 100% of the mice (Fig. 2).

Results

Opiates

The opiates were tested first without the intervention of opiate antagonists to establish a base line for the subsequent evaluation of interactions between the drugs. Figure 2 shows the percentage of mice showing the Straub reaction after injection with opiates. On the basis of their ED50 levels, the drugs appear to be from most to least potent as follows: oxymorphone, phenazocine, (−)-*trans*-I, morphine, meperidine, and codeine. The 100% level was not achieved with codeine, meperidine, and (−)-*trans*-I. One of twenty-six mice died within 25 min of injection with codeine 128 mg/kg. This mouse was discarded from the experiment.

Opiate antagonists with fixed doses of opiates

Table 1 shows the potency of seven opiate antagonists in counteracting the Straub reaction produced by morphine. The higher the dose of morphine, the higher the dose of antagonist necessary to counteract the Straub reaction. The antagonists had the same rank-order potency against morphine 16 and 32 mg/kg.

Table 2 shows the potency of opiate antagonists in counteracting the Straub reaction produced by fixed doses of several opiates. The doses of antagonists effective against meperidine were less than those effective against the other two opiates. This seems to be attributable to the fact that the dose of meperidine by itself was less effective in producing the Straub reaction than were the doses of the other test opiates by themselves. The various antagonists were equiactive against morphine and phenazocine within limits expected from errors of measurement. The rank-order coefficients of correlation for the ED50 values in each of the three columns were equal or greater than 0·95: 0·97 for meperidine versus morphine, 0·95 for meperidine versus phenazocine, and 0·99 for morphine versus phenazocine. Nalorphine and naloxine were about as active against their parent opiates as against

TABLE 3. *Potency ratios*

Strong antagonists	Isomerism		Potency ratios for different tests		
	Optical	Geometric	Rotarod	Hypoactivity	Lethality
Naloxone	(−)		8,100	7,500	>11,000
Cyclazocine	(−)	*cis*	57	760	10,000
Cyclazocine	(−)	*trans*	12	270	4,800
Cyclorphan	(±)	*cis*	50	790	5,700
Cyclazocine	(±)	*cis*	39	370	3,600
Cyclazocine	(±)	*trans*	—	—	2,800
Levallorphan	(−)		320	770	2,610
Nalorphine	(−)		840	290	2,200

Weak antagonists	Isomerism		Potency ratios for different tests		
	Optical	Geometric	Rotarod	Hypoactivity	Lethality
Pentazocine	(−)	*cis*	7·3	5·1	64
I	(−)	*cis*	7·1	4·1	68
I	(±)	*cis*	18	4·2	55
Pentazocine	(±)	*cis*	5·8	7·8	37
I	(+)	*cis*	5·5	6·4	—
Cyclazocine	(+)	*cis*	1·5	8·3	21
Pentazocine	(+)	*cis*	6·3	8·9	27
Cyclazocine	(+)	*trans*	<0·9	<1·9	<5·3

Data for rotarod, hypoactivity, and lethality are from work referred to in **Methods** section. The ED50 value for each drug in each test was divided by its ED50 for antagonizing the effects of morphine on the Straub reaction (Table 2). The greater the potency ratio, the more active the drug as an antagonist in comparison with its other effects. The drugs are listed in descending order of potency as morphine-antagonists.

other opiates. The ED50 values for nalorphine were 0·23 mg/kg against morphine 16 mg/kg (parent), 0·094 against meperidine 64 mg/kg, and 0·27 against phenazocine 1 mg/kg. Against approximately equiactive doses of opiates, the ED50 values for naloxone were 0·008 mg/kg with oxymorphone 0·5 mg/kg (parent) and 0·006 mg/kg with meperidine 64 mg/kg. Against the lowest doses of opiates that produced the reaction in all the mice, the ED50 values for naloxone were 0·025 with oxymorphone 1 mg/kg (parent) and 0·021 with phenazocine 1 mg/kg. Against (−)-*trans*-I 4 mg/kg, the ED50 for naloxone was 0·031.

Table 3 shows the potency of the antagonists in several different tests in comparison with their potency as opiate antagonists. To facilitate the analysis, the drugs were grouped into two categories: strong and weak antagonists. The potency ratios indicate that the drugs were more active in antagonizing the Straub reaction than in impairing rotarod performance or in producing hypoactivity and death. In contrast with the strong antagonists, the weak antagonists counteracted the Straub reaction at doses closer to those that produced other effects, with the exception that in the rotarod test the potency ratio of one of the strong antagonists, (−)-*trans*-cyclazocine, was lower than that of one of the weak antagonists, (±)-*cis*-I. Potency ratios similar to those shown in Table 3 can be obtained by using the data for either meperidine or phenazocine instead of the data for morphine. Likewise, similar potency ratios can be obtained by using convulsions as an index of toxic effects (Pearl *et al.*, 1968). Accordingly, whereas doses of the strong antagonists can be increased considerably above their antagonist ED50 values before toxic effects are encountered, this holds true to a lesser extent for the weaker antagonists.

Isomers

Included among the benzomorphan derivatives were four pairs of (−) and (+) isomers. The potency of these isomers and their racemates are compared in Table 4. The (−) isomers were far more active against the opiates than their corresponding (+) isomers. Larger differences in potency between the (−) and the (+) isomers were noted among the strong antagonists than among the weaker antagonists. Whereas *trans*-(−)-cyclazocine and *cis*-(−)-cyclazocine, two potent antagonists, were more than 600 times as active as their (+)-isomers, *cis*-(−)-pentazocine and *cis*-(−)-I, two weak antagonists, were less than 17 times as active as their (+) isomers. On the average the (−) isomers were about twice as potent as their racemates.

TABLE 4. *Potency ratios for the activity of isomers against opiates*

Opiate antagonist	Meperidine	Opiate Morphine	Phenazocine
cis-(−)-Cyclazocine	500	620	620
cis-(±)-Cyclazocine	200	230	210
trans-(−)-Cyclazocine	1,300	1,300	890
trans-(±)-Cyclazocine	940	670	590
cis-(−)-Pentazocine	17	5·2	8·9
cis-(±)-Pentazocine	5·8	2·8	3·2
cis-(−)-I	11	4·5	6·7
cis-(±)-I	3·2	4·0	2·9

Potency ratios were derived as follows: $\frac{\text{ED50 of (+) isomer}}{\text{ED50 of (−) isomer}}$

$\frac{\text{ED50 of (+) isomer}}{\text{ED50 of racemate}}$ ED50 values appear in Table 2.

Dose-response lines

Interactions between several opiates and opiate antagonists were examined further to gauge the nature of the antagonism (Figs. 3 and 4). The antagonism produced by naloxone and by nalorphine was surmountable until high doses of opiates and opiate antagonists were used. Up to that point, the dose-response lines appeared to be linear and approximately parallel to each other. Although this also appeared to hold for (−)-*cis*-pentazocine, increments in the dosage of this antagonist produced a relatively marked displacement of the dose-response lines to the right. The antagonism produced by (−)-*cis*-cyclazocine was surmountable in only a relatively narrow range of doses. The dose-response lines for (+)-*cis*-cyclazocine, (+)-*cis*-pentazocine, and atropine were linear and parallel within only a very narrow range of doses. Of the antagonists tested, naloxone and nalorphine, and perhaps (−)-*cis*-pentazocine, appeared to act like competitive antagonists. To determine the extent to which this was so, the data for these antagonists were analysed further.

"pAx" values

The values for naloxone, nalorphine, and (−)-*cis*-pentazocine (Fig. 3) were replotted on log-probit paper and fitted by a series of parallel lines. Sample data for phenazocine with naloxone are illustrated in Fig. 5. If the effects of a dose of opiate antagonist appeared to be insurmountable, the values for that dose were not replotted and were not given any weight in estimating the slope of the lines. Next,

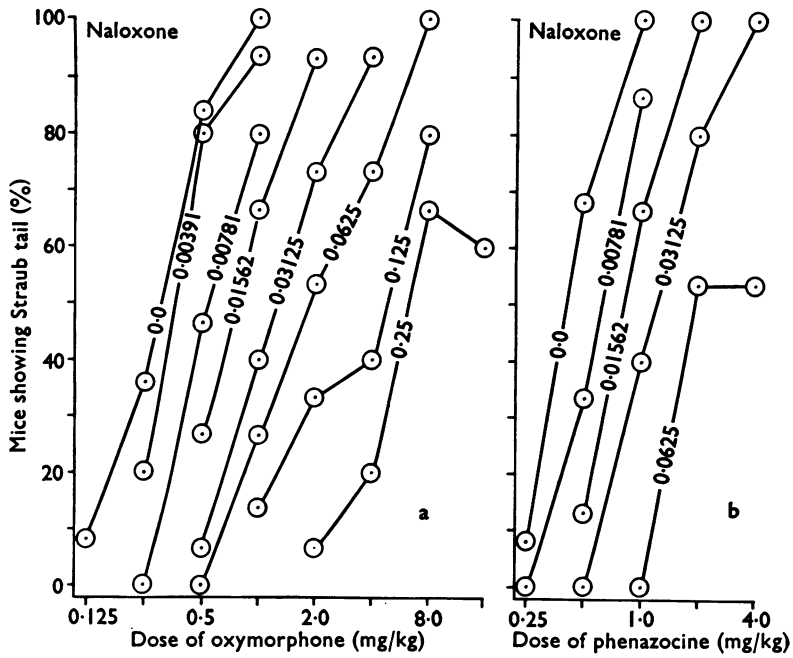

FIG. 3. The legend to this figure is shown on page 232.

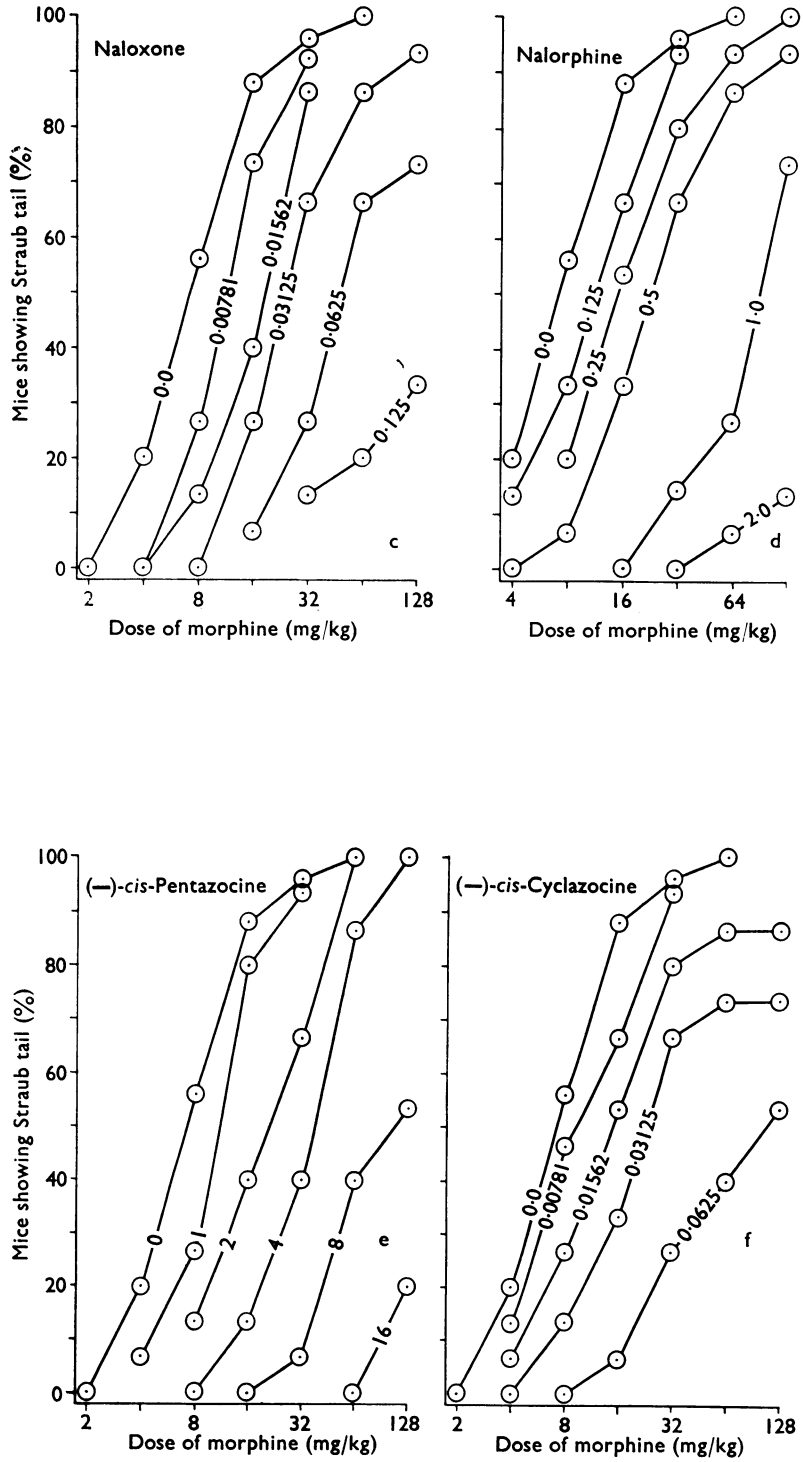

FIG. 3 (See also page 231). Percentage of mice showing the Straub reaction in the presence of opiates alone and in the presence of antagonists. The opiates and their doses are shown on the abscissa (log scales). The antagonists and their doses (mg/kg) are shown within the figures.

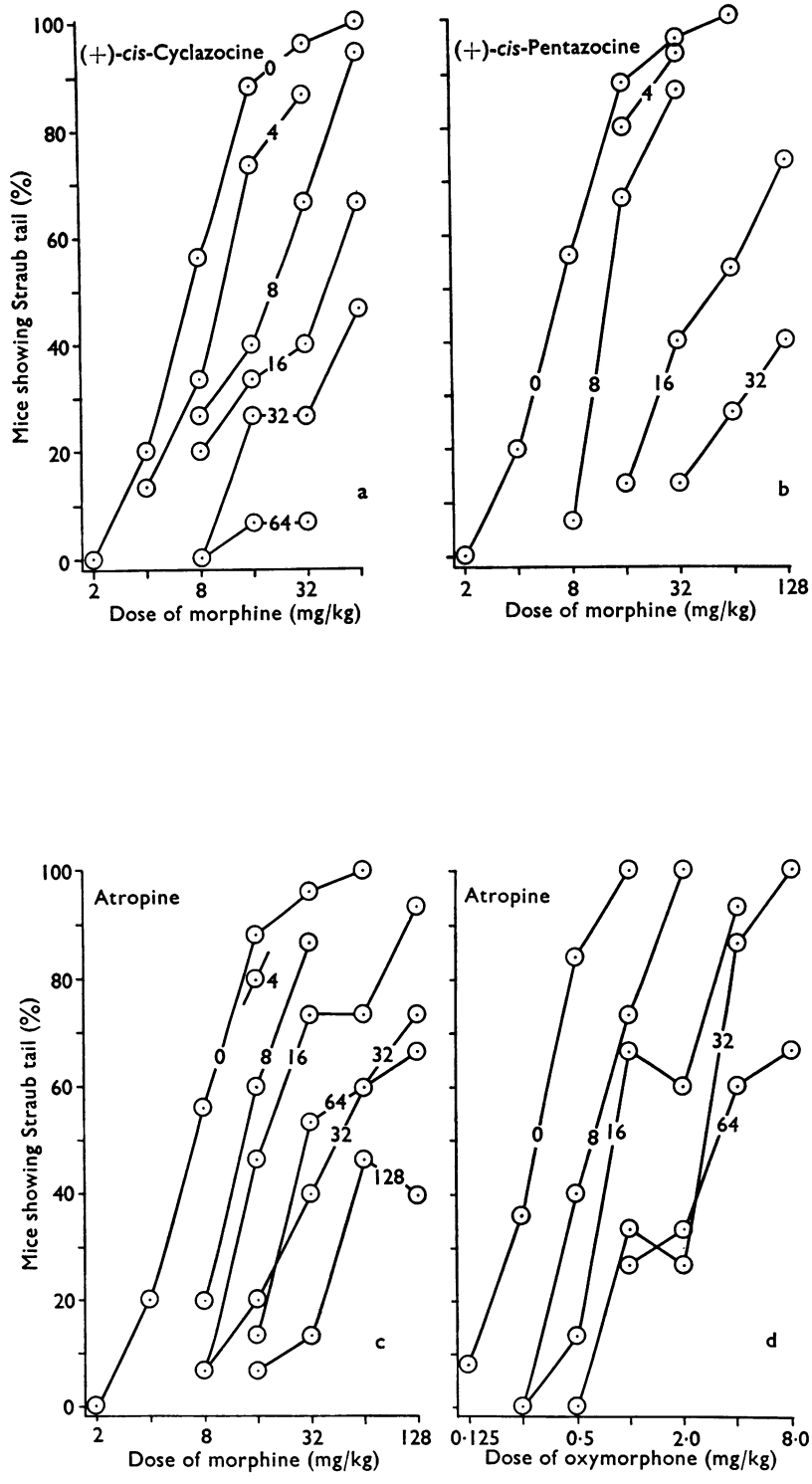

FIG. 4. Percentage of mice showing the Straub reaction in the presence of opiates alone and in the presence of antagonists. Doses are in mg/kg. Compare plots with those for Fig. 3.

ED50 values were calculated for each opiate alone and in the presence of different doses of the antagonist. Dose ratios (x) are presented in Table 5. Figures 6 and 7 show "pA_2" values derived by plotting log (x-1) against N, the negative logarithm of the molar dose the mice received on a weight basis. The "pA_{10}" values were derived by regression analysis. Table 6 shows the "pA_2" "pA_{10}" values.

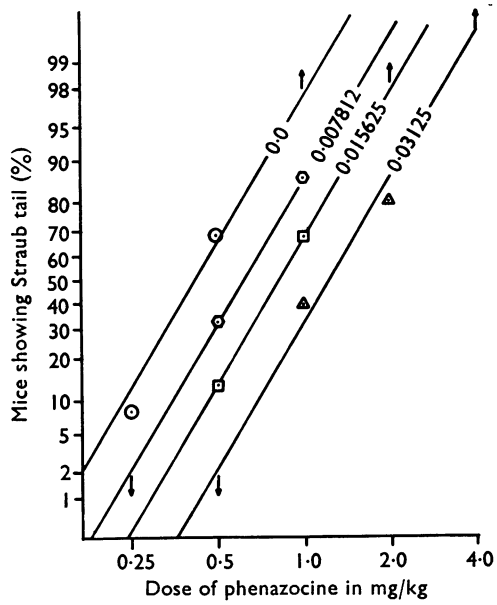

FIG. 5. Log-probit plot for Straub reaction produced by phenazocine alone and in the presence of naloxone. Arrows show doses that produced the reaction in 0 and 100% of the mice. Other details as in Fig. 3b, which shows the semilog plot for the data.

TABLE 5. *Dose ratios for the Straub reaction produced by opiates in the presence and absence of opiate antagonists*

Opiate	Opiate antagonist	Dose (mg/kg)	ED50 for Straub tail (mg/kg)	Slope function: ED50/ED16	Dose ratio (x)
Oxymorphone	Naloxone	0·0	0·292	1·8	—
		0·0039	0·362	1·8	1·2
		0·0078	0·566	1·8	1·9
		0·0156	0·759	1·8	2·6
		0·031	1·30	1·8	4·4
		0·0625	1·96	1·8	6·7
Phenazocine	Naloxone	0·0	0·416	1·6	—
		0·0078	0·620	1·6	1·5
		0·0156	0·820	1·6	2·0
		0·031	1·22	1·6	3·0
Morphine	Naloxone	0·0	7·29	2·2	—
		0·0078	11·8	2·2	1·6
		0·0156	17·5	2·2	2·4
		0·031	27·0	2·2	3·7
Morphine	Nalorphine	0·125	11·0	2·2	1·5
		0·25	16·3	2·2	2·2
		0·5	24·7	2·2	3·4
Morphine	(−)-*cis*-Pentazocine	1·0	10·8	2·2	1·5
		2·0	19·7	2·2	2·7
		4·0	34·8	2·2	4·8

The " pA_2 " values for naloxone with oxymorphone, phenazocine, and morphine were about the same (5·3 to 5·5). The differences between the " pA_2 " and " pA_{10} " values (about 0·9) were close to the theoretical value (0·95) for competitive

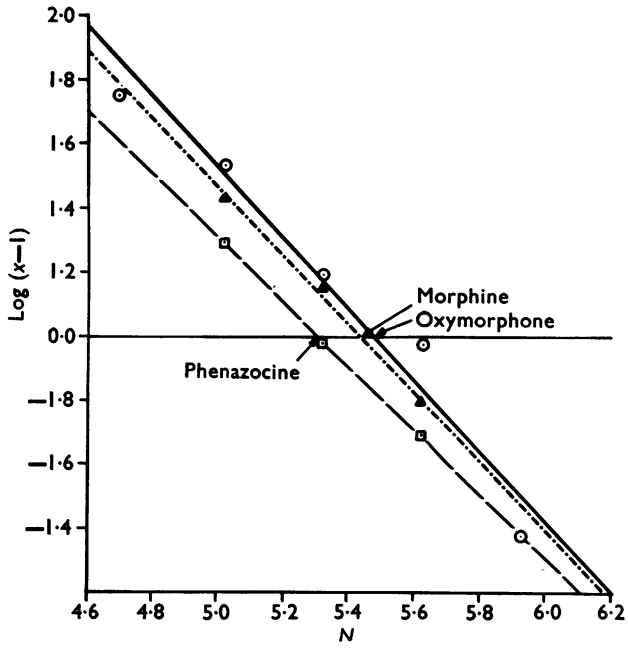

FIG. 6. Relationship between dose ratios and dose of naloxone for the Straub reaction produced by opiates. Points were fitted with straight lines by the method of least squares. Arrows indicate " pA_2 " values for naloxone with the three opiates. Abscissa: N, the negative logarithm of the molar dose of naloxone injected per 100 g of body weight. Ordinate: log (x-1), x being the dose ratio shown in Table 5.

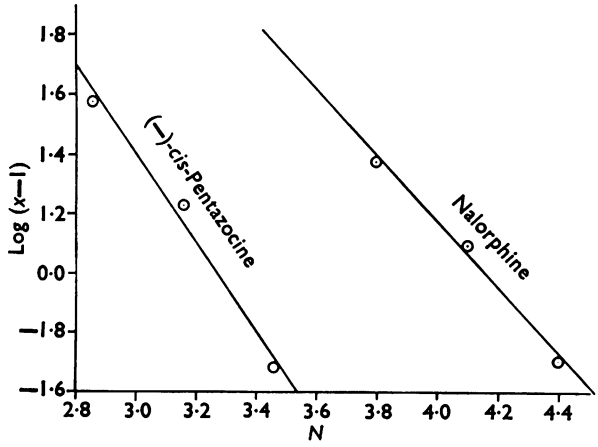

FIG. 7. Relationship between dose ratios and doses of antagonists for the Straub reaction produced by morphine. Antagonists are identified within the figure. Other details as in Fig. 6.

antagonism. This was also true for nalorphine with morphine. That is, the slopes of the lines for these antagonists with the opiates were about 45°. The differences between the "pA_2" and "pA_{10}" values (0·7) for (−)-*cis*-pentazocine with morphine appeared to be below the theoretical value for competitive antagonism.

TABLE 6. *"pAx" values for opiates with antagonists for Straub tail reaction*

Antagonist	Opiate	"pA_2"	"pA_{10}"	"pA_2"–"pA_{10}"
Naloxone	Oxymorphone	5·5	4·6	0·9
Naloxone	Phenazocine	5·3	4·4	0·9
Naloxone	Morphine	5·4	4·5	0·9
Nalorphine	Morphine	4·2	3·3	0·9
(−)-*cis*-Pentazocine	Morphine	3·3	2·6	0·7

Discussion

The generality of the results can be gauged by comparing them with previous ones in animals and in man. Table 7 shows the rank-order potency of the opiates in several tests. Inspection of the ranks suggests that the Straub tail values are reproducible and are correlated with those for other tests.

Table 8 shows the potency of sixteen antagonists in counteracting the Straub reaction produced by meperidine in mice and their potency in counteracting the inhibitory effects of meperidine in the tail-flick test in rats. Comparison of the results indicates that the values in the two tests are highly correlated (rho = 0·94). The rank-order potency for antagonists in counteracting the effects of opiates in man appears to be as follows: naloxone, levallorphan, and nalorphine (Foldes, Swerdlow & Siker, 1964). Similarly, the rank-order potency for antagonists in precipitating

TABLE 7. *Rank-order potencies of opiates in different tests*

Drug	Mice					Rats	Humans
	Straub A	Straub B	Phenyl-quinone B	Rota-rod B	Hot plate C	Tail-flick D	Analgesia E
Oxymorphone	1	1	1	1	–	–	1
Phenazocine	2	2	3	2	–	1	2
(−)-*trans*-I	3	3	2	3	–	2	–
Morphine	4	4	4	4	1	3	3
Meperidine	5	5	5	5	2	4	4
Codeine	6	6	6	6	3	5	5

Sub-headings under the species refer to the Straub tail reaction in mice, abdominal constrictions produced by phenylquinone in mice, ability of the mice to stay on a rotating rod, reactions of mice on a hot plate, reactions of rats to radiant heat focused on their tails, and the relief of pain in humans. Capital letters designate the studies from which the potencies were derived: A, Present study; B, Pearl *et al.*, 1968; see also Siegmund, Cadmus & Lu, (1957); Blumberg, Wolf & Dayton (1965); C, Eddy, Halbach & Braenden (1956); D, Archer *et al.* (1964); Grumbach & Chernov (1965); Tullar *et al.* (1966); E, review of literature by Lasagna (1964). Ranks in the body of the table are based on parenteral doses in mg/kg of the free base. Ranks in rodents are based on ED50 values and those in man on doses of drugs that give about the same degree of relief of pain. Unavailability of a rank estimate is indicated by a dash (−).

withdrawal symptoms in addicts appears to be as follows: naloxone, (±)-*cis*-cyclazocine, levallorphan, nalorphine, and (±)-*cis*-pentazocine (Fraser & Rosenberg, 1964 ; Martin, Fraser, Gorodetzky & Rosenberg, 1965 ; Jasinski, Martin & Haertzen, 1967). These results for man agree with those for rodents (Table 8).

The (−) isomers of four benzomorphan derivatives were much more potent in antagonizing the Straub reaction than their (+) isomers and on the average about twice as potent as their racemates. The activity of the isomers, especially that of the *cis* isomers, seemed to be consistent with Pfeiffer's (1956) generalization: the lower the effective dose of a drug, the greater the difference in the pharmacological effects of the optical isomers. This suggests that the degree of geometric conformation at the site of action is greater with potent drugs than with less potent drugs. In the present study, the lower the effective dose of the antagonist, the greater the difference in the antagonist potency of the optical isomers. This seems to hold not only for the antagonist potency of benzomorphans (Archer *et al.*, 1964 ; Tullar *et al.*, 1967) but also for several agonist-like effects of the benzomorphans (Pearl *et al.*, 1968).

Consistent with previous findings (Archer & Harris, 1965 ; Pearl *et al.*, 1968), (−)-*cis*-I (C-5 ethyl homologue of 1-pentazocine) acted like an opiate antagonist, while its *trans* isomer acted like an opiate. Although the cis form rather than the *trans* form is more closely related sterically to the *cis*-fused B/C ring system of morphine (Fullerton, May & Becker, 1962), the unnatural *trans*-fused ring system may often afford better access to or fit on the receptor.

In the present study log (x-1) was plotted against N (where x is the dose ratio obtained by dividing the ED50 for the opiate alone by that for the opiate in the presence of the antagonist and N is the negative logarithm of the molar dose of the antagonist on a weight basis). Naloxone acted like a competitive antagonist inasmuch as the slopes of the regression lines for nalorphine against oxymorphone, morphine, and phenazocine were all about 45°. Likewise, nalorphine acted like a competitive antagonist against morphine. The "pA_2" value of 4·2 was close to

TABLE 8. *Potency of antagonists in counteracting the activity of meperidine in rodents*

Drug	Straub tail reaction in mice: ED50 (mg/kg)	Rat tail-flick test: ED50 (mg/kg)
Naloxone	0·006	0·004
(−)-*cis*-Cyclazocine	0·006	0·006
(±)-*cis*-Cyclazocine	0·015	0·019
Levallorphan	0·020	0·052
(−)-*trans*-Cyclazocine	0·024	0·005
Cyclorphan	0·024	0·034
(±)-*trans*-Cyclazocine	0·034	0·014
Nalorphine	0·094	0·13
(−)-*cis*-I	0·63	3·1
(−)-*cis*-Pentazocine	0·66	0·9
(±)-*cis*-Pentazocine	1·9	3·9
(±)-*cis*-I	2·2	11
(+)-*cis*-Cyclazocine	3·0	2·5
(+)-*cis*-I	7·0	20
(+)-*cis*-Pentazocine	8·8	14
(+)-*trans*-Cyclazocine	>32	19

Results for the rat tail-flick test were taken from Archer *et al.* (1964), Tullar *et al.* (1967), and Pearl *et al.* (1968). All drugs were injected subcutaneously.

those previously reported for nalorphine in different tests in mice (Cox & Weinstock, 1964). Similarly, others have noted that nalorphine acts like a competitive antagonist against various opiates in rats (Grumbach & Chernov, 1965 ; Blane *et al.*, 1967).

Any conclusions, however, must be tempered by the limitations noted by others when measures of quantal responses in intact animals are substituted for measures of graded responses in isolated organs (Gaddum, 1957 ; Loewe, 1957 ; Cox & Weinstock, 1964 ; Grumbach & Chernov, 1965). Indeed, nalorphine has sometimes failed to act like a competitive antagonist in intact animals (Martin, 1967). A limitation encountered in the present study was that the doses of the antagonists could be increased, at best, only about 8 to 16 times above their ED50 levels. At higher doses the antagonism was not surmountable. Perhaps this was so, at least in part, because very high doses of opiates by themselves were no longer as effective as moderately high doses in producing the Straub reaction. Interestingly, at low concentrations naloxone, nalorphine, and levallorphan act like competitive antagonists with opiates *in vitro* and at higher concentrations they do not do so (Cochin, Spivak & Lipper, 1967).

Five drugs failed to fulfil criteria for competitive antagonism. The first drug, atropine, has no close structural similarity with the opiates and is not potent in blocking the Straub reaction. Although (+)-*cis*-cyclazocine and the (−) and (+) isomers of *cis*-pentazocine have structural similarities with opiates, the three opiate antagonists are not potent in blocking the Straub reaction. However, the fifth drug, (−)-*cis*-cyclazocine, was quite potent in blocking the Straub reaction.

The Straub tail test in mice seems to be useful as a preliminary test for studying structure-activity relations among opiates and opiate antagonists in intact animals. Irrespective of the degree of specificity of the test, it appears to be useful for estimating the potency of opiates and of opiate antagonists and for detecting differences in the dose-response lines of various antagonists.

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