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COMPARATIVE EFFECTS OF ANALGESICS ON PAIN THRESHOLD, RESPIRATORY FREQUENCY AND GASTROINTESTINAL PROPULSION

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In the rat, the ratio of the analgesic to the respiratory depressant potency was the same for morphine, codeine, diamorphine, methadone, dipipanone, piperidylisomethadone, phenadoxone, dextromoramide, and propoxyphene. The relative respiratory depressant activity of pethidine tended to be less, but the difference was not significant. The ratio of the analgesic dose to the dose preventing transport of a charcoal meal in the rat was about the same for morphine, codeine, pethidine, methadone, phenadoxone, dimethylthiambutene, and propoxyphene; the relative activities of these compounds in inhibiting the peristaltic reflex of the isolated guinea-pig ileum were also similar. However, because of differences in the slopes of regression lines in the charcoal meal test, some compounds (for example, morphine) had a greater effect on gastrointestinal propulsion than others (for example, pethidine) when given at moderate analgesic dose levels.

In studies of the effects of intracisternal morphine in the rat, effects on the spinal reflex of the tail were to some extent dissociated from effects on the threshold for a squeak response. Further, the delaying of transport of a charcoal meal paralleled depression of respiratory rate, and this is evidence for the participation of a central as well as a peripheral action in the effect of morphine on the gastrointestinal tract. The delay in propulsion was reduced by nalorphine and increased by atropine and two general anaesthetic substances, but was unaffected by a number of other pharmacological agents.

The elevation of pain threshold with morphine and allied drugs is commonly accompanied by a reduction in breathing and a delay in gastrointestinal transport (Krueger, Eddy, and Sumwalt, 1941; Reynolds and Randall, 1957). The relationship between the doses causing these effects is known to a limited extent for a few compounds in man, but such comparisons as have been carried out in laboratory animals have often been unsatisfactory as quantitative studies because of inadequate investigation of the dose/response relationships or of failure to compare the pharmacological effects in the same species under strictly comparable conditions. Comparative observations upon the rat are presented in this paper. The compounds examined represent several series of analgesics, with effects resembling those of morphine even to their being antagonized by nalorphine. The comparisons were usually made after subcutaneous or intravenous administration, but morphine was also given intracisternally to see if a central site was important for these effects. The comparative effects of analgesics on the peristaltic reflex of the isolated guinea-pig ileum and the effects of various pharmacological agents on gastrointestinal propulsion in untreated rats and rats treated with morphine are also reported.

METHODS

Analgesia and Respiratory Frequencies. — Pain thresholds estimated by the heat and pressure methods of Green and Young (1951) were measured 30 min. after the injection of the analgesic substances subcutaneously at two or more concentrations into groups of ten or twenty rats aged three to four weeks. Activities relative to that of morphine, tested on the same occasion, were determined from the regression on log dose of the probit of the proportion of rats in the group with pain thresholds at least twice that of a control group injected with saline. Respiratory frequencies were measured immediately following the

pain threshold determinations by recording the pressure changes in a tambour held against the belly of the animal (Green, 1953a). Respiratory depressant potencies were estimated from the regression of frequency on log dose; no significant differences in slopes were detected.

Gastrointestinal Effects.—The charcoal meal test introduced by Macht and Barba-Gose (1931) for examining cathartics and used by Karr (1947) in testing the effects of morphine, methadone and pethidine, was investigated. The rats were prepared in two ways. Those animals referred to as "fasted" were given no solid food for a day but water *ad libitum* up to 1 hr. before the test. The "dieted" rats received a liquid diet of 5% glucose (w/v) in protein hydrolysate and water *ad libitum*, for two days until 1 hr. before the test. Animals were housed in cages with wire mesh bottoms. The test meal, given by stomach tube, was 0.3 or 0.5 ml. of a smooth suspension containing charcoal, wheat flour and water in the proportions by volume of 1:2:6. At stated time intervals afterwards, the rats were killed by a blow on the head and the proportion of the intestine traversed was measured immediately.

Control studies (Fig. 1) showed that the rate of propulsion was most rapid in the first part of the small intestine and was uniform for the first 10 min. It then progressively decreased. The mean proportion of the small intestine traversed in 10 min. was significantly greater in the dieted rats (0.58, s.e. ±0.018) than in the fasted rats (0.42±0.024). The 10 min. period was chosen as most suitable for studying drug effects. Because of a small day-to-day variation in controls it has been usual to express the proportions of the intestine traversed as percentages of the mean in a concurrent control group as the first step in analysing the results.

Drugs were normally given subcutaneously or intravenously. In the main comparisons, they were injected subcutaneously 20 min. before the charcoal meal as the analgesic effects of these drugs reached a maximum between 20 and 30 min. after subcutaneous injection in young rats. Most of the drugs included in Fig. 2 were compared in a single experiment on groups of ten rats; five animals were given each dose on one day and five on another. The results for codeine and propoxyphene [(±)-4-dimethylamino - 3 - methyl - 1:2 - diphenylbut - 2 - yl propionate] are from separate experiments using groups of six rats.

In the second comparison of morphine, methadone and pethidine, groups of sixteen animals were given morphine sulphate, 0.153, 0.613, 2.5, 10 or 40 mg./kg;

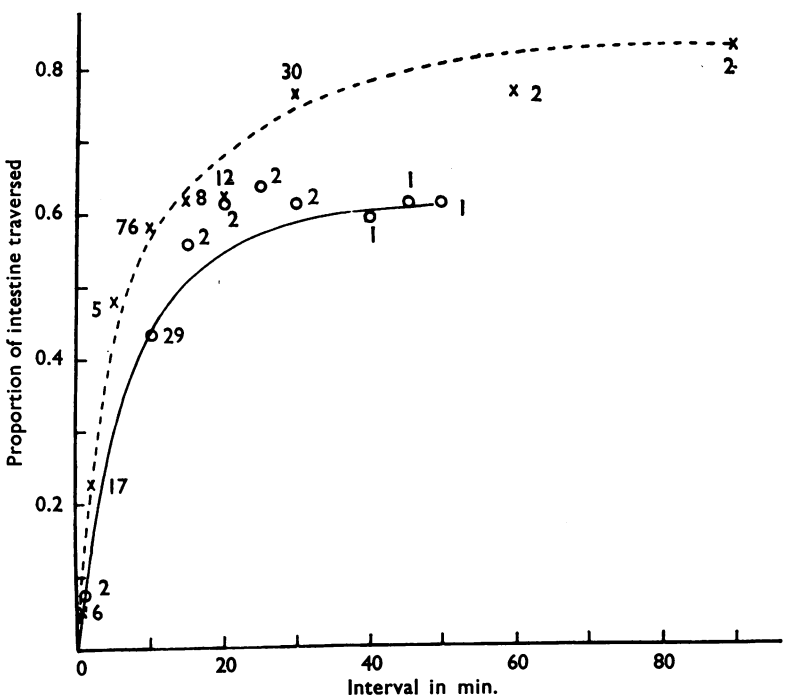

FIG. 1.—The proportion of the small intestine traversed at intervals after administering a charcoal meal in fasted (O—O) and dieted (X - - - X) rats. The number of measurements is indicated by the numeral beside each point.

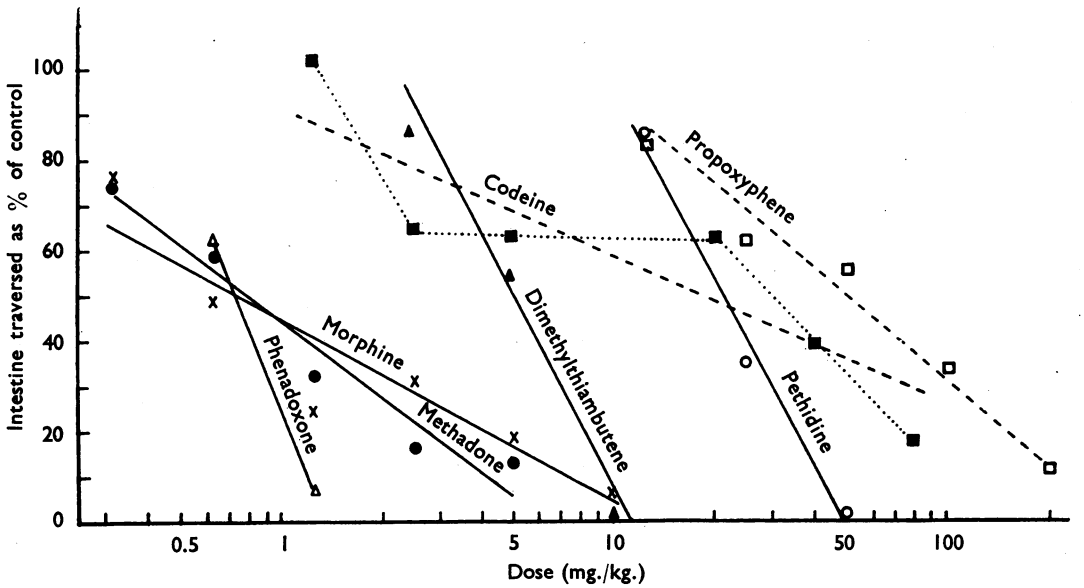

FIG. 2.—The effect of analgesics, injected subcutaneously, on the propulsion of a charcoal meal in rats. The regression on log dose of the proportion of the small intestine traversed by the charcoal in 10 min., as % of the mean in controls. All the compounds except codeine and propoxyphene were compared in the same experiment.

methadone hydrochloride, 0.078, 0.313, 1.25, 5 or 20 mg./kg.; or pethidine hydrochloride, 6.25, 12.5, 25 or 50 mg./kg. The pain thresholds of the animals were measured immediately before they were killed. The rats used in these experiments were three to five weeks old. Saline was injected in controls.

Intracisternal Tests.—The injections were made under short ether anaesthesia using a needle with a bent tip, as described for mice by Lockett and Davis (1958). The needles were 201 gauge with a polythene tube sheath that left only the last 2.5 mm. of the needle exposed. The charcoal meal was administered 20 min. after the morphine, by which time the effects of the ether had largely passed off and the morphine depression was about maximal. Pain thresholds and respiratory frequencies were estimated immediately before killing the rats for measurements of intestinal propulsion. Sixteen rats were used at each dose, four on each of four test occasions. The labels of doses were coded.

Peristaltic Reflex.—The stretch reflex of isolated guinea-pig ileum suspended in oxygenated Tyrode solution at 37° was induced by raising the intraluminal pressure by 2 to 3 cm. water and longitudinal contractions and volume changes recorded by the method of Trendelenburg. The response was determined before and 3 min. after each of a series of increasing doses of the analgesics; the dose interval approximated to $\sqrt{10}$. The log dilutions (pD values) causing slight (±) and nearly complete (+++) inhibition were determined for the longitudinal muscle contractions and the volume change.

RESULTS

The Association of Analgesic and Respiratory Depressant Actions in the Rat

In the rat, the same quantitative relationship between analgesic activity and depression of respiratory frequency was found for morphine and some thiambutene derivatives (Green, 1953a), and by Green and Ward (1956) for morphine and morpheridine (morpholinoethylnorpethidine). Similar comparisons of other drugs are summarized in Table I. (Some of these results were reported to the British Pharmacological Society by Green and Young in 1950.) No significant differences were found between the relative analgesic and respiratory depressant properties of morphine, diamorphine, codeine, pethidine, methadone, phenadoxone, dipipanone, piperidylisomethadone (2-methyl-3:3 diphenyl-1-piperidino-hexan-4-one hydrobromide), dextromoramide and propoxyphene. There was a tendency for the respiratory depressant effect of pethidine to be less ($P=0.07$).

TABLE I

RELATIVE ANALGESIC AND RESPIRATORY DEPRESSANT POTENCIES AND THE PROBABILITIES OF THE RATIOS OF THESE ACTIVITIES BEING UNITY

Activities were usually derived by pooling the estimates from two or more comparative tests; n=the total number of animals given the test substance; an equal number received morphine. The analgesic potencies are from the pressure thresholds for a squeak response; the potencies from heat reaction times were the same.

Drug	n	Relative Activity		A/R	Probability of A/R=1·0
		Analgesia (A)	Respiration (R)		
Morphine sulphate ..	—	1·0	1·0	1·0	—
Codeine phosphate ..	30	0·20	0·22	0·91	>0·5
Diamorphine (base)..	30	7·14	6·49	1·10	>0·5
Pethidine hydrochloride ..	128	0·44	0·31	1·43	0 07
Methadone hydrochloride ..	80	2·48	2·59	0·95	>0·5
Dipipanone hydrochloride ..	80	2·32	2·15	1·08	>0·5
Piperidylisomethadone hydrobromide ..	80	0 93	1·06	0 88	>0·5
Phenadoxone hydrochloride ..	80	5·75	5·15	1·11	>0·5
Dextromoramide (base)	60	13·68	16·72	0·82	>0·5
Propoxyphene hydrochloride ..	110	0·114	0·108	1·05	>0·5

Delay in Gastrointestinal Propulsion

Analgesics Given Subcutaneously.—The effects on the propulsion of a charcoal meal are compared in Table II and Fig. 2. The proportions of the small intestine traversed within 10 min. are expressed as percentages of the mean in the control group. Analysis showed homogeneity of variances, the overall mean value being about 850, but there was a tendency for the variance to decrease at the higher levels of effect. The values were related linearly to log dose for each compound, deviation not being significant at the 5% level. Analysis by the χ^2 test of observations on the five compounds investigated within the same experiment (Table II) showed no significant difference between the slopes for morphine and methadone ($P=0.2$) or between those for pethidine, phenadoxone, and dimethylthiambutene ($P=0.6$), but the slopes for morphine and methadone were significantly less steep ($P<0.001$) than those for the other three compounds. The doses reducing intestinal transport by 50% were calculated and doses stopping the entry of charcoal into the intestine were derived by extrapolation from the dose/response lines. The latter dose of morphine, methadone, pethidine, and dimethylthiambutene was in each instance about four times the corresponding analgesic ED50, and this indicated that the two effects were related. The relative analgesic and intestinal depressant activities of pethidine, dimethylthiambutene and phenadoxone were similar at all levels of response, and these compounds only affected intestinal transport at doses higher than those needed for a marked analgesic action. On the other hand, morphine and methadone delayed intestinal transport at doses lower than those causing appreciable analgesia in the rat.

In a second comparison of morphine, methadone, and pethidine, measuring analgesic and intestinal effects in the same dieted rats, the use of larger groups of animals and a greater dose range allowed more precise definitions of dose/response relationships. The slopes in the charcoal meal tests differed significantly from each other, being, for morphine, -32 ± 3.0; methadone, -43 ± 2.6; and pethidine, -89 ± 12.2. No differences were detected between the slopes in the analgesic tests. The mean and 95% limits of the doses in mg./kg. reducing propulsion by 50% were 1.22 (0.84 to 1.76), 0.95 (0.75 to 1.21), and 18.2 (14.7 to 22.4) for morphine sulphate, methadone hydrochloride, and pethidine hydrochloride respectively, and the corresponding estimates for each analgesic ED50 were 4.4 (3.2 to 6.2), 2.4 (1.4 to 3.9), and 14.0 (9.4 to 20) respectively.

The results of separate experiments on propoxyphene and codeine are included in Fig. 2. The regression for propoxyphene was linear, the slope being nearly as steep as that for pethidine. The values for codeine were not readily fitted by a straight line, and the dotted

TABLE II

THE EFFECTS OF SUBCUTANEOUS INJECTIONS OF ANALGESICS IN THE CHARCOAL MEAL TEST

The analgesic doses shown are those earlier found equivalent to 3 mg./kg. morphine sulphate in elevating the threshold to pressure: (a) Green and Young (1951); (b) Green (1953). Doses in mg./kg. 95% limits in parentheses.

Drug	Charcoal Meal Test			Equivalent Analgesic Doses
	Slope±s.e.	Dose for 50% Reduction	Dose for 100% Reduction	
Morphine sulphate	−41± 5·6	0·72 (0·50–1·04)	12	3·0
Methadone hydrochloride	−55± 9·7	0·79 (0·58–1·08)	6	1·3 (1·2–1·4) (a)
Pethidine ,, ...	−139±21·7	21·5 (19·0–24·4)	50	11·0 approx. (a)
Phenadoxone ,, ...	−189±43·2	0·73 (0·67–0·79)	1·5	0·60 (0·47–0·78) (a)
Dimethylthiambutene hydrochloride, ..	−143±21·7	5·16 (4·30–6·21)	12	2·8 (2·4–3·3) (b)

line in Fig. 2 indicates that there seem to be two distinct components in its effect. Each drug largely prevented gastric emptying when about four times the analgesic ED50 was given, as has already been noted with the other compounds examined. Propoxyphene showed a constipating effect in the rabbit (Robbins, 1955).

Analgesics Given Intravenously.—Although the above tests did not discriminate between effects on the pylorus and effects on the propulsive mechanism of the small intestine, the latter were shown to play a major part in tests where the drugs were injected intravenously after a time which had been shown in control studies (Fig. 1) to be sufficient to allow charcoal to enter the small intestine. In an experiment using groups of five dieted rats, in which intravenous doses were given 2 min. after the charcoal, the mean percentage of the small intestine traversed in a total of 10 min. was 43 ± 5.4 (s.e.) in saline controls, 20 ± 7.1 after 2 mg./kg. morphine sulphate, 19 ± 8.2 after 2 mg./kg. methadone hydrochloride, 16 ± 6.1 after 20 mg./kg. pethidine hydrochloride, 7.6 ± 6.4 after 1 mg./kg. phenadoxone hydrochloride and 9.4 ± 8.6 after 5 mg./kg. dimethylthiambutene hydrochloride. The values for the groups given analgesics were not significantly different from one another, but all were significantly reduced below the control value. Though in this experiment the distance travelled by the charcoal at the time the analgesics were injected was not determined, in comparable tests the mean percentage travel in 17 rats killed 2 min. after giving the meal was 22 ± 4.0. Even though the control rate of propulsion was somewhat slower in this experiment than usual (10 min. travel 43%, compared with the overall control mean of 58%), it was clear that the intravenous doses of these analgesics, especially phenadoxone and dimethylthiambutene, must have abolished or nearly abolished propulsion. The intravenous doses were all between a quarter and a half of those which largely abolished propulsion in the subcutaneous tests (Fig. 2).

Peristaltic Reflex

Schaumann, Giovannini, and Jochum (1952) showed that the relative activities of morphine, pethidine, and the optical isomers of methadone in depressing the peristaltic reflex of isolated guinea-pig ileum were somewhat similar to their relative analgesic potencies in mice and rats. Several estimates of the log dilutions for causing slight and nearly complete inhibition of the reflex are summarized in Table III. Analysis of the tests on morphine, pethidine, methadone, and phenadoxone showed that the variance of the *p*D values was the same (0.04) for each drug at each level of effect, whether determined from the response of the longitudinal muscle or the volume change. Further, no difference was found

TABLE III

INHIBITION OF THE PERISTALTIC REFLEX OF ISOLATED GUINEA-PIG ILEUM

Means causing slight (±) and nearly complete (+++) inhibition of the reflex longitudinal muscle response and volume change; the potency ratios are derived from the mean of these *p*D values; n = number of observations on ileum from different guinea-pigs.

Compound	n	*p*D±	*p*D+++	Potency Ratio
Morphine sulphate	8	7·31	6·83	1·0
Codeine phosphate	3	6·53	5·20	0·063
Pethidine hydrochloride	6	6·44	5·84	0·12
Methadone hydrochloride	6	7·89	7·34	3·53
Phenadoxone hydrochloride	5	8·18	7·44	5·51
Dimethylthiambutene hydrochloride	2	7·45	6·75	1·07
Propoxyphene hydrochloride	3	6·75	5·83	0·16

TABLE IV

INTRACISTERNAL MORPHINE IN RATS

Effects at 30 min. after various doses in 0·05 ml. saline. Analgesia: nos. with squeak response thresholds and heat reaction times for tail flick response greater than twice control mean, and nos. with thresholds outside control 95% limits; respiratory frequency; % intestine traversed by a charcoal meal in 10 min.

	Control Mean±s.d.	Dose (mg./kg.)				
		0·005	0·02	0·08	0·32	1·28
No. in group	16	16	15	16	16	16
Pain threshold (cm. Hg)	8·9±2·4					
No. above 2 × control	—	1	4	13	16	16
,, 95% limit	—	1	7	15	16	16
Heat reaction time (sec.)	3·9±1·2					
No. above 2 × control	—	0	1	1	12	16
,, 95% limit	—	0	2	2	14	16
Respiratory frequency						
Mean/min. ±s.d.	294±72	291±72	213±114	210±95	136±48	106±43
No. below 95% limit	—	0	5	7	11	14
% intestine traversed						
Mean ± s.d.	39±13	41±14	27±20	26±17	15±18	3±3
No. below 95% limit	—	0	4	4	10	16

between the *p*D values for the longitudinal muscle response and those for the volume change, and the differences between the values producing the two levels of effect were alike for the four drugs. The means of these two *p*D50 values were determined for each test and found to have an overall variance for all compounds of 0.027. The potency ratios in Table III were determined from the antilog of the difference between the mean of these values for each compound and that for morphine. These ratios are similar to the relative potencies determined in the intact rat, though two species and entirely different test conditions are concerned in such a comparison. The effects of all the analgesics on the peristaltic reflex were inhibited by nalorphine at 0.1 μg./ml.

The Effects of Morphine Injected Intracisternally

The effects in the rat of various doses of morphine injected intracisternally, on (*a*) the threshold pressure on the tail required to cause a squeak response, (*b*) the reaction time for a tail flick response when radiant heat was focused on the tail, (*c*) respiratory frequency, and (*d*) transport of a charcoal meal, are summarized in Table IV and Fig. 3. Several points of interest arise. First, all the effects occurred at much lower doses than with the subcutaneous route, for example, 50 to 100 times less for the pressure squeak response. Second, heat reaction times for the tail flick tended to be least affected, the ED50 being at least four times the ED50 for the pressure squeak response, whereas the difference was trivial by subcutaneous injection (Green and Young, 1951). This suggested that when given intracisternally morphine did not attain such a high concentration at the lower levels of the cord concerned with the tail flick reflex as it did in some parts of the brain. Third, the depression of respiration relative to the elevation of the squeak threshold tended to be greater after intracisternal than after subcutaneous injection; after intracisternal injection the concentration of morphine would be greatest near the medulla. Fourth, the effects on respiration and intestinal transport appeared at the same dose and increased roughly in parallel. The action of the intracisternal morphine on intestinal transport was so great as to indicate that a central action must be concerned in its effects on the alimentary tract when the drug is administered by other routes.

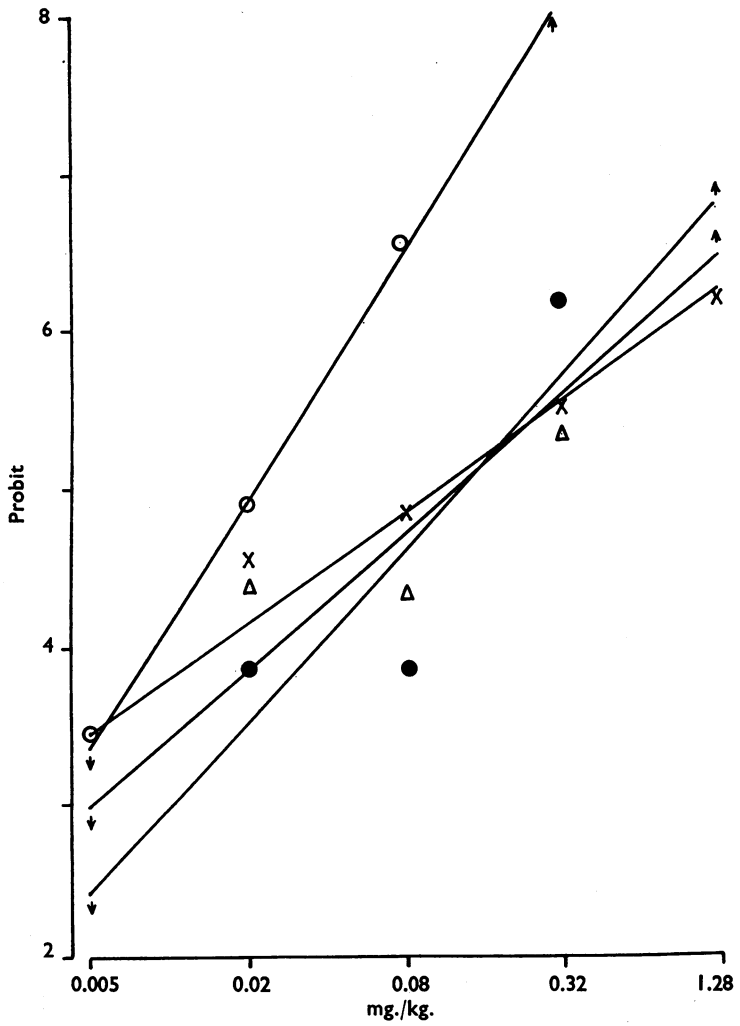

FIG. 3.—The effects of intracisternal morphine in rats. The regression on log dose of the probit of the number of animals in the group with values outside control 95% limits (see Table IV). O, pressure threshold for squeak response; ●, heat reaction time for a tail flick response; X, rate of breathing; Δ, % small intestine traversed by a charcoal meal.

Effects of Various Pharmacological Agents in the Charcoal Meal Test when Given Alone or in Combination with Morphine Sulphate

These effects are summarized in Table V and Fig. 4. Atropine reduced the passage of the charcoal but to a much smaller degree than morphine; its effect was synergistic with that of morphine. Pentobarbitone sodium also retarded propulsion but only in amounts that were near those producing anaesthesia; this substance and tribromoethyl alcohol greatly increased the effect of morphine even in amounts that when given alone had insignificant effects. Large doses of adrenaline delayed propulsion and were synergistic with morphine. No effect was produced by large doses of papaverine, dibenamine (50 mg./kg.), or the antihistamine *trans* - 1 - *p* - chlorophenyl - 1 - pyrid - 2′ - yl - 3 - pyrrolidin-1″-ylprop-1-ene hydrochloride (Green, 1953b) either alone or in combination with morphine.

Nalorphine reduced intestinal transport, though to a smaller extent than did morphine, and it antagonized the effect of morphine on propulsion (Fig. 4). The best antagonism of the effect of 10 mg./kg. morphine sulphate was seen with 10 mg./kg. nalorphine, the effect on propulsion then being less than with either compound alone; after 10 mg./kg. morphine and 30 mg./kg. nalorphine the effect was similar to that with large doses of nalorphine alone.

Discussion

The ratios of analgesic activity to respiratory depressant activity by the methods used here were the same for each of several analgesics representing diverse chemical groups. The only possible exception was pethidine, though even here the difference was not significant ($P=0.07$). An indirect comparison of the effects for morphine and the isomers of dextromoramide by de Jongh and van Proosdij-Hartzema (1957) revealed no dissociation of the analgesic and respiratory depressant properties, though in a later paper using the same results for respiratory depression but different estimates of analgesia obtained by another method, Janssen, Jagenueau, van Proosdij-Hartzema and de Jongh (1958) claimed that dextromoramide and a new pethidine analogue

TABLE V

CHARCOAL MEAL TEST: EFFECTS OF VARIOUS COMPOUNDS ALONE AND IN COMBINATION WITH MORPHINE OR METHADONE

Intestine traversed measured 10 min. after charcoal administration, 30 min. after the analgesics (s.c.) and at various intervals after treatment with the compound under test (s.c.). Means for groups of six rats and standard errors. Dose in mg./kg. unless stated. Morphine given as sulphate, 10 mg./kg., methadone as hydrochloride, 5 mg./kg. Bromethol was a 66·6% w/w solution of tribromoethyl alcohol in amylene hydrate diluted with saline.

Expt. No.	Compound	Dose	Interval (min.)	Mean % Intestine Traversed ± s.e. (or Range in Parentheses)	
				+ Saline	+ Morphine
1	Saline		15 and 30	64± 2·8	27 ±7·4
	Adrenaline tartrate	0·5 twice	15 and 30	32± 3·5	12 ±2·9
	Pentobarbitone sodium	20	30	26± 8·3	2·9±0·53
2	Saline		60	65± 4·1	12 ±2·8
	Atropine sulphate	0·25	60	39± 5·7	5·0 (0 to 26)
	,, ,,	1·0	60	32± 5·8	3·7±0·6
	,, ,,	4·0	60	27± 5·2	1·8 (0 to 5)
3	Saline		60	43± 3·5	20 ±3·2
	Atropine sulphate	5	60	34± 8·3	3·3±0·7
	,, ,,	50	60	22± 8·2	5·3±4·5
4	Saline		60	49± 4·1	12 ±3·2
	Papaverine HCl	10	60	54± 4·5	10 ±4·4
	,, ,,	100	60	56± 9·0	15 ±5·1
	Antihistamine 405C49	20	60	52±13·4	8 ±3·6
5	Saline		60	41± 4·1	16 ±5·0
	Pentobarbitone sodium	10	60	50± 3·6	5·3±1·6
	,, ,,	20	60	49± 2·4	9·8±6·1
	Bromethol	0·2 ml.	60	36± 4·9	4·2±2·1
	Dibenamine	50 mg.	60	44± 3·9	16 ±4·9
				+ Saline	+Methadone
6	Saline		60	52± 4·1	0·83 (0 to 3)
	Atropine sulphate	1	60	23± 4·6	4·8 (0 to 11)
	,, ,,	10	60	27± 2·1	6·5±0·92
	,, ,,	100	60	6·3 (0 to 30)	1·1 (0 to 4)

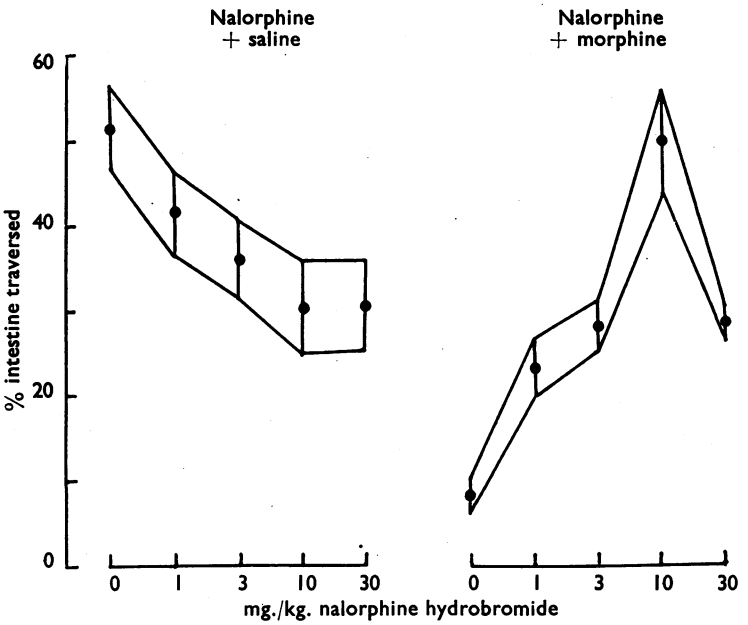

FIG. 4.—The percentage of small intestine traversed in 10 min. by a charcoal meal after nalorphine ± 10 mg./kg. morphine sulphate injected subcutaneously at the same time. Drugs given 20 min. before charcoal. Vertical lines represent s.e. (n=10 for each point).

had relatively less effect than morphine on respiration. In our experiments, the respiratory depressant activity of dextromoramide was proportional to its analgesic activity. Our experience has been that estimates of effective doses may vary significantly from day to day in different batches of rats, and that only direct comparative tests are safe in studies of this kind. There are few comparable results published for other species. Interpretations have often been misleading, since the analgesic activity in one species (usually the rat) has not uncommonly been compared with respiratory depressant action in another (usually the rabbit). Such comparisons as have been made in the dog are limited by the small numbers of animals used. The analgesics which have not caused respiratory depression to the same extent as morphine in the dog would seem to be those with which a stimulant or convulsant effect supervened before high levels of analgesia are attained, as for example with (+)-methadone (Luduena and Ananenko, 1950) and propoxyphene (Robbins, 1955; Green, unpublished observations). In general the present results in the rat are in accord with those in man.

For each of several analgesics the amount that largely stopped the entry of a charcoal meal into the small intestine was about four times the analgesic ED50 value, and where the slopes of regression lines relating log dose to the proportion of the intestine traversed by the charcoal were parallel, the effect was proportional to the analgesic activity in different compounds. However, with morphine and to a lesser degree methadone, these slopes were shallow by comparison with those for pethidine, dimethylthiambutene and phenadoxone; in contrast with this group, morphine and methadone delayed transport of the charcoal at doses below those causing appreciable analgesia. A difference of this type between the effects of equiactive analgesic doses of morphine, methadone, and pethidine was reported by Karr (1947), but the relation of dose to effect was not studied. Tests of constipating effect in the rat, by counts of faecal pellets (see for example Schaumann *et al.*, 1952) are for comparative purposes open to the objection that since the counts are necessarily taken long after the main part of the analgesic action has passed off, the relative effects of compounds will depend as much on duration of action as on intensity of effect. A charcoal meal test has been used in mice by Janssen and Jageneau (1957) and Janssen *et al.* (1958), who gave analgesics 1 hr. before the charcoal and killed 2 hr. later to count the number of appendices blackened by the charcoal. Though this again may test the duration of action of the compounds rather than measure their activity, the various ratios of the ED50 obtained in this way to those for analgesic effects show a twenty-fold spread for different compounds, the distinction between morphine, methadone, and pethidine being small.

Vaughan Williams and Streeten (1950) made an extremely valuable study of the effects of morphine, methadone, and pethidine on fluid transport in cannulated Thiry-Vella loops in dogs. Since they did not make comparable observations on the analgesic potency of these compounds but relied on estimates reported for the rat, it is appropriate to compare their findings with those on analgesic activities in the dog obtained in this laboratory (Green, 1953a and unpublished observations). Their threshold subcutaneous doses for reducing fluid transport were 0.03, 0.08, and 1.7 mg./kg. for morphine, methadone, and

pethidine, respectively ; our estimates of the ED50 for elevating pain threshold by 100% in the dog are 1.0, 0.65, and 15 mg./kg. respectively. Hence for morphine, methadone, and pethidine the very approximate ratios of the equipotent doses on the bowel to the equipotent analgesic doses are 1:4:4; these ratios have wide limits, those of the analgesic comparisons alone being of the order of 60 to 180% of the means (Vaughan Williams and Streeten (1950) report the ratios of equipotent doses on the bowel of the dog to equipotent analgesic doses in the rat as 1:5:75, but from their observations the ratios are 1:5:7.5). The major difference between the results in the dog and those reported here for the rat is that only in the former were the regressions of effect on log dose the same for morphine, methadone, and pethidine. It may be significant, in relation to this difference, that the results for the dog involve only the small intestine, whereas in those for the rat an action on the pylorus may also be concerned. It has been shown by administration of analgesics intravenously that a major part of the delay in propulsion of the charcoal meal in the rat occurs in the small intestine, but the importance of an action on the pylorus has not been assessed. Two components seem to be concerned in the dose/response relationship of codeine, and it is possible that more detailed studies might reveal two components in the effects of other analgesics in this test.

Nalorphine was the only one of several pharmacological agents examined which antagonized the action of morphine in charcoal meal tests in rats. Atropine increased the effect of morphine in these tests, though Vaughan Williams and Streeten (1951) found that atropine antagonized the effect of moderate, but not large, doses of morphine on fluid transport in cannulated Thiry-Vella loops in conscious dogs.

The evidence concerning the possible sites of action of morphine on the alimentary tract has been reviewed by Krueger *et al.* (1941) and Reynolds and Randall (1957). The powerful inhibitory effects of several compounds on the peristaltic reflex of the isolated guinea-pig ileum described here, like similar findings of Schaumann *et al.* (1952) and Schaumann (1954 and 1955), emphasize the importance of a peripheral mechanism. The retardation of the propulsion of a charcoal meal caused by low intracisternal doses of morphine shows that a central site of action must be concerned in the rat. Similar findings were reported by Margolin (1954) for intracranial injections of morphine in the mouse, and mention was made of comparable effects in the rat and guinea-pig. Both central and peripheral sites are clearly of importance in the complex actions of morphine on the alimentary tract, but the importance of a central action in the corresponding effects of allied drugs has not been established.

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