Brit. J. Pharmacol. (1956), **11**, 27.

ANALGESIC ACTION IN A SERIES OF *N*-SUBSTITUTED ETHYL 4-PHENYLPIPERIDINE-4-CARBOXYLATES

BY

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(RECEIVED OCTOBER 8, 1955)

Following the synthesis of pethidine by Eisleb and Schaumann (1939), a large number of its analogues have been tested for analgesic potency. The present series, synthesized by R. F. Anderson, P. M. Frearson, and E. S. Stern in the Research Department of J. F. Macfarlan and Co. Ltd., comprises derivatives in which the *N*-methyl group of pethidine has been replaced by a tertiary amino alkyl group. The substances tested are listed in Table I.

METHOD

Analgesia was measured in rats by the pressure method of Green and Young (1951), using apparatus different in some ways from that described. The pressure was measured with a mercury manometer, the bore of which was 3 mm. It was transmitted to the tail by a strip of wood (12×4×5 mm.) which was cemented to the plunger head of the large vertical syringe and applied to the tail at right angles about 1 cm. from the tip. The small horizontal syringe was operated by hand and was fitted with a spring under compression between the barrel and plunger head, to ensure a rapid release of pressure. A narrow capillary tube with a valve in parallel was later introduced between the two syringes; this restricted the rate of increase of pressure without unduly affecting the rate of release. The syringes were of 20 ml. and of 2 ml. capacity and their cross-sectional areas (and thus the applied forces) were in the ratio 6.5:1. A smaller ratio required more effort from the operator, and a steady increase of pressure was more difficult to achieve. In practice, pressure was increased until the rat squeaked, when the height of the mercury column was noted and the pressure released.

Green and Young abandoned quantitative measurements of analgesia in favour of an all-or-none method. This may involve waste of information, and we persisted in using the technique quantitatively. The difficulty is that, with effective doses of an analgesic, some rats fail to respond to any pressure, and an arbitrary value has to be allotted to obtain a mean value for a group. To avoid this we have taken as an index of analgesia the ratio

$$\frac{\text{Pressure required to induce a squeak before injection}}{\text{Pressure required to induce a squeak after injection}}$$

If a rat did not squeak with a pressure four times the control figure the index was taken as 0, which, unlike infinity, can be included in a mean value for a group. The index was calculated from the pressure measured at 15, 30, and 45 min. after injection. The substances tested did not differ markedly in duration of action, and we therefore calculated the mean of these three values to give a single index for the response of each rat. The inclusion of a control pressure might be expected to render the index independent of the dimensions of the apparatus and of the size of the rat's tail. Green and Young (1951) showed that the variation of the control pressure between rats of the same age was no greater than between different trials on the same rat. On some occasions, therefore, we have used, as numerator in each index, the mean control pressure of the rats used on that occasion. At other times the individual control pressures have been used.

All substances tested were injected subcutaneously in 4 ml. saline/kg. body weight.

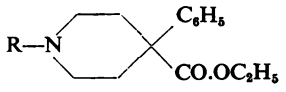
RESULTS

The first compound in the series, TA1 (Table I), was soon found to be considerably more potent than pethidine, and doses of 5 mg./kg. given to 50 g. rats frequently induced complete analgesia.

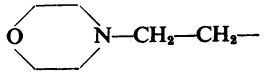
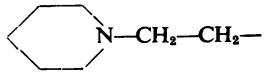
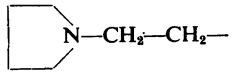
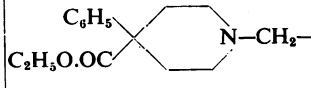
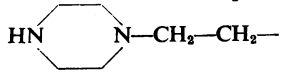
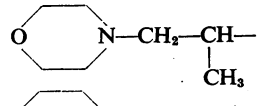
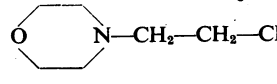
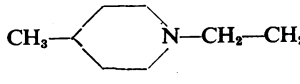
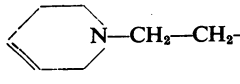
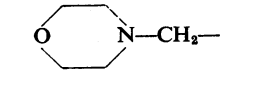
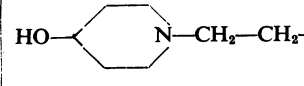
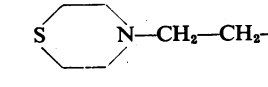
The use of rats on more than one ocasion did not reveal the development of tolerance, and the cross-over technique was used for a direct comparison of TA1 and pethidine. Two separate tests were carried out, each comprising a (2+2) dose, 4-way cross-over test in which 24 rats were used. In the first test the rats were divided into four groups of six (body weight evenly distributed, but otherwise at random), each group receiving in turn pethidine, 4 mg./kg. and 8 mg./kg., and TA1, 1.5 and 3 mg./kg. This test was so arranged that the groups received the four doses in a different order, and there was a 2- or 3-day interval between each dose. In the second test the doses were increased to 8 and 16 mg./kg. pethidine, and to 2.5 and 5 mg./kg. TA1; each rat was given these doses in a different order—using all the 24 possible ways—at 3-day intervals.

TABLE I

RELATIVE ANALGESIC ACTIVITIES OF ETHYL 4-PHENYL-PIPERIDINE-4-CARBOXYLATES



(In pethidine: R is CH_3)

TA No.	R	Salt	Relative Activity
1		2 HCl	100
2		2 ,,	<5
3		2 ,,	—
4		2 ,,	—
5	Cl—CH_2—CH_2—	1 ,,	<5
6		3 ,,	—
7		2 ,,	40
8		2 ,,	15
9		2 ,,	—
10		2 ,,	—
13	$(HO.CH_2.CH_2)_2N$—CH_2—CH_2—	2 ,,	—
14		2 ,,	?5–10
16		2 ,,	—
18		2 ,,	33

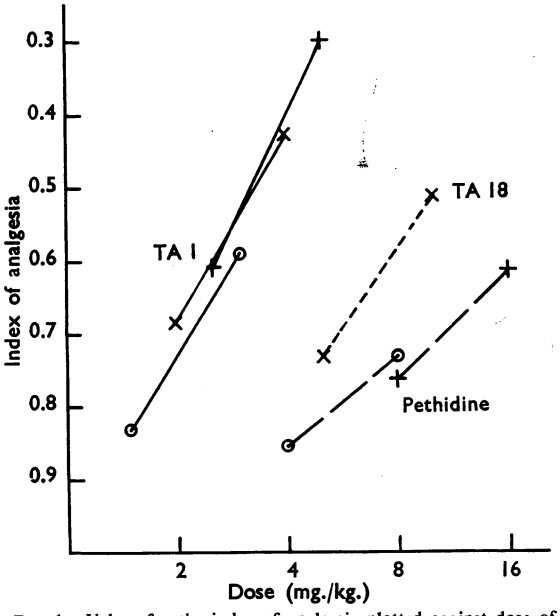


FIG. 1.—Values for the index of analgesia plotted against dose of drug. Each point is the mean from 24 rats obtained in a (2 + 2) dose 4-way cross-over test. All points from the same test are indicated by the same symbol. Points from the first test comparing TA1 and pethidine are marked ⊙; points from the second test of TA1 and pethidine are marked +; and those from the test comparing TA1 and TA18 are marked ×. The pairs of points for the two doses of a drug used in any one test are joined by straight lines.

In the first test the analysis of variance showed the log dose response slopes to be significantly different, so that the potency ratio (TA1 to pethidine), calculated from this test to be 4.8, has no absolute value. This was confirmed by the second test, where larger doses gave the higher potency ratio of 6.5 (fiducial limits 4.51 and 9.47, $P=0.95$). Here the difference in slopes was also apparent (see Fig. 1), but owing to the greater error variance (Table II) the difference was not quite significant at the 5% level.

Most of the other compounds in the series were without appreciable activity. TA3, TA6, TA13, and TA16 produced no effect when tested in doses of at least 50 mg./kg. TA2 had some effect: the mean index in a group of six rats given 80 mg./kg. was 0.62. TA5, 100 mg./kg., produced a definite response (the mean index for six rats was 0.48).

Solubility limited the doses of TA4, TA9, and TA10, and no appreciable analgesia was seen. For six rats given 22.5 mg./kg. TA4 the mean index was 0.84; for five rats given 25 mg./kg. TA9 it was 0.97; for five rats given 37.5 mg./kg. TA10 it was 0.95. TA14 was only available in very small quantity; 30 mg./kg. was injected in four rats and

TABLE II

COMPARISON OF TA1 AND PETHIDINE: ANALYSES OF VARIANCE

Source of Variation	d f.	Sum of Squares	Variance	Variance Ratio	P
First Comparison					
Between drugs	1	0·4874	0·4874	32·9	<0·1%
Regression ..	1	0·6767	0·6767	45·7	<0·1%
Deviation from parallelism ..	1	0·1395	0·1395	9·4	<1·0%
Between days ..	3	0·1399	0·0466	3·15	<5%
,, rats ..	23	0·9332	0·0406	2·74	<1%
Error	64*	0·9472	0·0148		
Total ..	93*	3·3239			
Second Comparison					
Between drugs	1	1·3090	1·3090	30·4	<0·1%
Regression ..	1	1·2308	1·2308	28·6	<0·1%
Deviation from parallelism ..	1	0·1528	0·1528	3·5	>5%
Between days ..	3	0·0504	0·0168	0·391	—
,, rats ..	23	0·9013	0·0392	0·912	—
Error	66	2·8358	0·0430		
Total ..	95	6·4801			

* Observations could not be made on one rat after the first two days of the first test. Values calculated to avoid biasing the means for the appropriate group on each of the two remaining days were inserted in the results. The degrees of freedom associated with error were consequently reduced to 64 in this test.

there was probably some effect (mean index 0.73). TA7 and TA8 were both found to be effective analgesics. The relative activities shown in Table I are based on a test in which TA7 (12.5 mg./kg.), TA8 (25 mg./kg.), and TA1 (2 mg./kg. and 4 mg./kg.) were given to four groups of five rats. The test was repeated 7 days later, the drugs being interchanged so that each dose was given to ten rats in all.

So far the only substances in this series to show appreciable activity contained the morpholino ring, and the oxygen atom appeared essential. It therefore became of considerable interest to obtain and test the analogous compound containing sulphur in place of oxygen. This compound, TA18, was found to be quite active and was compared with TA1, in a (2+2) dose 4-way cross-over test similar to the second of the tests in which TA1 and pethidine were compared. In this test the slopes for TA1 and TA18 were parallel (the appropriate variance was actually less than the error variance) and TA1 was 3.03 (limits 2.46 and 3.74, $P=0.95$) times as active as TA18.

Pethidine, like morphine, is antagonized by nalorphine, and we found that 2 mg./kg. of nalorphine was effective in antagonizing the analgesic action of 4 mg./kg. of TA1 when the two drugs were given simultaneously; however, this dose of nalorphine was much less effective against 10 mg./kg. of TA1; 24 mg./kg. of nalorphine exerted an analgesic action of its own (Table III).

TABLE III

NALORPHINE ANTAGONISM TO THE ANALGESIC ACTION OF TA1

Group of 6 Rats	Dose mg./kg.		Mean Index of Analgesia
	Nalorphine	TA1	
I	—	4	0·18
II	2	4	0·83
III	12	—	1·01
I	24	—	0·67
II	—	10	0·19
III	2	10	0·58

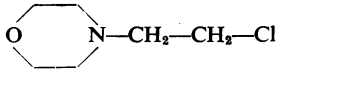
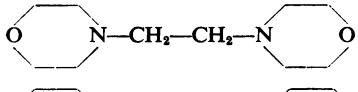
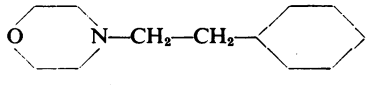
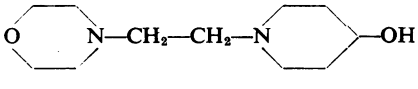
The tests shown in the last 3 rows were done 7 days after the first tests using the same 3 groups of rats.

The substituent groups in this series of compounds are introduced into pethidine in a similar position to that in which the allyl group is introduced into the morphine molecule to give nalorphine. We therefore investigated the possibility of antagonism of TA1 by TA2 or TA3. Nine rats were injected with 5 mg./kg. TA1. In three rats this was preceded by 50 mg./kg. TA2; analgesia was complete, the mean index being 0. In another three rats the dose of TA1 was preceded by 50 mg./kg. TA3; the index was 0.04. The remaining three rats were given saline before the injection of TA1, and here the index was 0.17. The effect of TA2 or TA3 with TA1 is, if anything, additive rather than antagonistic.

The importance of the morpholino ring when attached to the pethidine nucleus lent some interest to several simpler compounds containing this ring (see Table IV), but it was not surprising to find that they were not effective. In a dose of 100 mg./kg., TA11 did raise the pain threshold, but 5 of the 6 rats given this dose were dead the day

TABLE IV

SIMPLE DERIVATIVES OF MORPHOLINE TESTED

TA11	
TA15	
TA17	
TA19	

after the test. Smaller doses did not produce an analgesic effect, but induced a permanent disability, causing the rats to "waltz." This effect has previously been observed by Goldin, Noe, Landing, Shapiro, and Goldberg (1948) with several compounds, including TA11. The other compounds of this group, TA15, TA17, and TA19, showed no analgesic activity in doses of 100 mg./kg.

Toxicity of Morpholinoethylnorpethidine Hydrochloride (TA1)

Acute.—Unlike pethidine, TA1 did not induce convulsions in mice, but the Straub tail reaction was frequently seen. There was some irritability, followed by prostration. Death occurred after a period of marked respiratory depression. The intraperitoneal LD50 in 10–20 g. mice was 118 mg./kg. The toxicity increased sharply with dose, and the fiducial limits for this estimate of LD50 were 113 and 123 mg./kg. In heavier mice the toxic dose, expressed in mg./kg., was somewhat lower. Duguid and Heathcote (1940) and Gruber, Hart, and Gruber (1941) give intraperitoneal LD50's for pethidine of 125 and 147 mg./kg. A small number of mice were injected subcutaneously with doses up to 600 mg./kg. of TA1 without any deaths.

In rats injected subcutaneously the individual lethal doses varied from 50 to 200 mg./kg. The LD50 was between 70 and 100 mg./kg. (In our experience the subcutaneous LD50 of pethidine is about 200 mg./kg., the figure quoted by Barlow and Lewis, 1951.)

Subacute.—Three groups of 10 rats were weighed and given daily subcutaneous injections over 11 days. One group received TA1, 20 mg./kg., the second pethidine, 60 mg./kg., and the third group was injected with saline only. One rat in the TA1 group died on the 10th day, while only four of the rats receiving pethidine survived to the end of the test. The rats were weighed and killed on the 12th day. The mean weight gain of the surviving rats in each group was 26.7 g. with TA1, 26 g. with pethidine, and 26 g. in the controls. The liver, kidneys, spleen, heart, lungs, and brain of the surviving rats were examined histologically. No pathological changes were observed in these tissues. The rat in the TA1 group which died on the 10th day of the test was also examined and showed the following pathological changes in sections of lung tissue: alveoli oedematous and engorged with blood; gross generalized congestion; diffuse haemorrhages; haemorrhagic fluid in bronchioles. No pathological changes were noted in the liver, kidneys, spleen, or heart.

Discussion

We have found the pressure method of Green and Young to be a useful technique for observing the effects of analgesic drugs. The "index of analgesia" described in the section on method is a convenient extension of the original procedure.

Tests on small groups of rats soon showed that morpholinoethylnorpethidine was superior to any of the other compounds in the series, and relatively little effort was expended on these others. It was rather surprising that the presence of oxygen or sulphur in the heterocyclic ring should be so important for analgesic action. The compound with the morpholino ring is 3–7 times more potent than the parent compound, pethidine, whereas compounds without the oxygen (or sulphur) atom, but otherwise closely similar, are practically inactive despite the fact that nearly all the pethidine structure remains intact.

If the chain of carbon atoms linking the nitrogen atoms of the two rings in TA1 is lengthened (TA8) or shortened (TA14) there is a considerable reduction of activity. Branching also reduces activity (TA7), though not sharply. TA4 comprises essentially two molecules of pethidine joined together, but has little or no analgesic activity.

The use of a cross-over technique for comparing analgesics is not usual. The possibility of tolerance developing has presumably kept others from using this procedure although tolerance would not necessarily invalidate a symmetrical assay. In the present tests no evidence of tolerance was observed and the practical advantages would seem to outweigh theoretical disadvantages. In the first of the two cross-over tests in which TA1 and pethidine were compared the design was poor. The effects of the different doses were confounded with possible effects arising from the animals being housed in 4 separate cages: in addition all animals given a particular dose on the 2nd, 3rd, and 4th days of the test had a common dose history which was different from the histories of the rats given the other doses. This assay could therefore be misleading; but in fact the results agreed well with the second test, in which these faults were eliminated. The second design had the additional advantage that the doses were injected in irregular order down the list of rats; this eliminated the possibility of systematic effects due to the order of injecting and testing, and also made the actual testing of threshold more objective, since the observer did not remember at the time of testing

which dose each rat had received. This made it unnecessary to take special precautions to ensure that the observations were unbiased, which otherwise would have been very desirable because of the occasional difficulty in deciding the precise end-point.

When different substances are compared in a biological test it frequently happens that the dose-effect lines are not parallel. The errors of such tests are large, so that differences in slope are usually not significant; but with pethidine and morpholinoethylnorpethidine the difference in slope is sufficient to make it reasonably certain that the difference is real. This has the immediate disadvantage that it is not possible to state that TA1 is "R" times more potent than pethidine, but it also indicates that TA1 may be qualitatively as well as quantitatively a better analgesic than pethidine. It is generally believed that some of the analgesics used clinically produce a maximal effect larger than that produced by others, and it is possible that this difference is analogous to the difference of slope between TA1 and pethidine which we have observed in rats. There is thus some reason for supposing that TA1 may be more useful than pethidine in the treatment of severe pain. In rats the difference between the drugs is such that doubling a dose of TA1 is as effective as quadrupling the equi-active dose of pethidine.

The few toxicity tests that we have done provide some ground for supposing that the increase in potency seen with TA1 is not accompanied by as great an increase in toxicity. The tests in mice, though favourable to TA1, are probably not of much value, since the absence of marked convulsions with TA1 makes close comparison with pethidine irrelevant. In rats death follows a period of depression with both substances, and a comparison of toxicity has some value. The rather wide range of the individual lethal doses would require the use of a large number of animals to give a precise comparison; but our results suggest that TA1 is not three times more toxic than pethidine—and this is about the minimum value of the ratio of analgesic activity. The subacute toxicity test confirmed that TA1 is less than three times as toxic as pethidine, since, with this ratio of doses, pethidine killed 6 of 10 rats, and TA1 killed only 1 of 10, over the period of eleven days.

Summary

1. A series of compounds in which the *N* methyl group of pethidine is replaced by a tertiary amino alkyl group has been tested for analgesic activity in rats.

2. One of these, morpholinoethylnorpethidine, is considerably more potent than pethidine and its toxicity is not correspondingly high.

We wish to thank Dr. R. F. Ogilvie, Department of Pathology, Edinburgh University, to whom we are indebted for examining the sections, and Dr. A. H. B. Masson, who tested compounds TA13 and TA14.

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