RESEARCH PAPERS

THE INFLUENCE OF THREE PHENOTHIAZINE DERIVATIVES AND OF AMIPHENAZOLE ON THE ACTION OF METHADONE

STUDIES WITH TWO ALGESIMETRIC METHODS IN UNTRAINED HUMAN SUBJECTS

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The influence of the phenothiazine derivatives chlorpromazine, acepromazine and mepazine and of the phenylthiazole derivative amiphenazole on the pain-threshold-raising action of methadone is described. The tests were made in untrained human subjects by two algesimetric methods, one involving thermal stimulation of the skin, and the other mechanical stimulation of the finger nail-bed. The thermal method was reliable, whereas the mechanical one did not disclose analgesic potency. Chlorpromazine and acepromazine, proved to have analgesic activity but did not significantly increase the analgesic effect of methadone. Mepazine, which lacked analgesic activity, antagonised the methadone analgesia. Amiphenazole was found to exert an analgesic action by itself but it did not decrease the potency of methadone. The side-effects of methadone were increased by chlorpromazine and acepromazine, but were uninfluenced by mepazine and amiphenazole.

SEVERAL phenothiazine derivatives are known to produce a potentiation of the effect of analgesics and hypnotics. Chlorpromazine [2-chloro-10-(3′-dimethylaminopropyl)phenothiazine] was shown by Courvoisier, Fournel, Ducrot, Kolsky and Kretschet[1], to potentiate the analgesic effect of morphine in mice. This observation was confirmed by several clinical investigations[2–7]. Acepromazine [2-acetyl-10-(3′-dimethylaminopropyl)phenothiazine], in animal experiments with the radiant heat stimulation method of d'Amour and Smith, potentiated morphine as did chlorpromazine[8]. Mepazine [10-(1′-methyl-3′-piperidylmethyl)phenothiazine] also potentiated the analgesic activity of morphine studied by the hot-plate method in mice[9]. Some clinical observations seemed to confirm this finding. No experimental data were, however, presented[10]. Amiphenazole (2,4-diamino-5-phenylthiazole) is structurally unreleated to the aforementioned phenothiazine derivatives. This substance was introduced clinically to alleviate the respiratory depression, vomiting, drowsiness, depression of the cough reflex and even addiction of morphine[11]. Clinical trials showed that it did not reduce the analgesic action of morphine[12,13].

The present paper deals with the influence of these substances on the analgesic action of methadone. Previously we found that methadone produced a significant analgesic effect in untrained human subjects

studied by a thermal method, radiant-heat stimulation of the skin, and a highly probable analgesic effect tested by an electric method, electric stimulation of the tooth pulp[14].

We now compare the same thermal method with a mechanical method, pressure stimulation of the nail bed.

Material and Methods

Subjects. The experiments were made on 184 untrained, healthy male and female subjects of from 20 to 25 years. Each subject was used for one experiment only and was informed about its nature.

Thermal method. The radiant-heat stimulation method was used. The stimulus, which had a constant intensity of 300 millicalories/sq. cm./sec., was produced by a Hardy-Wolff-Goodell Dolorimeter (Williamson Development Co., West Concord, Mass.) and was applied to the unblackened skin of the forehead. The time required to reach the pain threshold was denoted as the *reaction time.* The analgesic effect is reflected in a prolonged reaction time.

Mechanical method. To obtain a mechanical pain stimulus, the apparatus described by Hardy, Wolff and Goodell[15], manufactured by Williamson Development Co., West Concord, Mass., was used. The apparatus consists of a plunger surrounded by a metal sleeve, within which is mounted a steel spring. The force in grams exerted on the finger nail-bed by the tip of the plunger was read from the scale of the instrument. The force was always applied to the base of the nail, and the subject was instructed to report the first pain sensation. Each measurement of the pain threshold was the mean of 10 readings, one on each finger. An analgesic action is reflected in an increase in the threshold value.

Drugs and dosage. Methadone hydrochloride, 6 mg., chlorpromazine hydrochloride (Hibernal, Leo) 15 mg., acepromazine hydrochloride (Plegicil, Pharmacia) 5 mg., mepazine acetate (Lacumin, Lundbeck) 15 mg., amiphenazole hydrochloride (Fenamizol, ACO) 7·5 mg., or, 1 ml. of saline were given either singly or in combination by intramuscular injection.

Performance of experiments. Threshold determinations were made 15–30 minutes before and 1 and 2 hours after drug administration. All subjects were instructed to rest in an armchair for 30 minutes before and during the experiment. Side-effects occuring during and after the experiment were noted by the subjects. The threshold determinations were carried out as double-blind tests; both methods were used simultaneously.

Statistical analyses. The analgesic effect was computed as the post-medication deviation from the pre-medication threshold value as follows.

In each test subject, X_0 denotes the pre-medication threshold value, and X_1 and X_2 the first and second post-medication threshold value, respectively. A value, Y, of the analgesic effect in each test subject is derived from the following formula: $Y = X_1 + X_2 - 2X_0$. Y represents

the response of each subject. On the basis of the Y values for the test subjects with the *same* medication, the mean, $\overline{Y}$, and the standard error of the mean were calculated.

The significance of the difference between the $\overline{Y}$ values for two medications compared was tested by *t* analysis. The degree of significance was estimated as follows:

Significant:	$P < 0{\cdot}001$: symbol +++
Highly probable:	$0{\cdot}001 < P < 0{\cdot}01$: symbol ++
Probable:	$0{\cdot}01 < P < 0{\cdot}05$: symbol +
Not probable:	$0{\cdot}05 < P$: symbol O

These symbols are used in Figures 1 and 2.

The means of X_0, X_1 and X_2 were computed for each drug treatment and were symbolised by $\overline{X}_0$, $\overline{X}_1$ and $\overline{X}_2$. In Figures 1 and 2, $\overline{X}_0$ was put at the origin. The deviations of $\overline{X}_1$ and of $\overline{X}_2$ from $\overline{X}_0$ were plotted in the Figures to show the variation in analgesic effect of the drugs and drug combination during the post-medication period. The total analgesic potency of each medication must be judged from the estimates of variance. The standard error of the $\overline{Y}$ values varied appreciably.

Each $\overline{Y}$ value and each curve represents 11–13 test subjects, except in the acepromazine experiments (Fig. 1B and 2B), where only 7 test subjects were available for each administration.

Results

Methadone and Chlorpromazine (Fig. 1A and 2A). Both methadone and chlorpromazine exerted an analgesic action tested by the thermal method. Their combined administration had a slightly greater effect, which differed highly probably from that of methadone, but not from that of chlorpromazine. With the mechanical method, only chlorpromazine produced a probable analgesic effect. The action of all other medications was nil.

Methadone and Acepromazine (Fig. 1B and 2B). Both methadone and acepromazine had an analgesic action tested by the thermal method. Their combined administration produced no increase in action. No effect was disclosed by the mechanical method.

Methadone and Mepazine (Fig. 1C and 2C). Only methadone had a significant activity, whereas neither mepazine nor the combination of the two drugs exerted any analgesic action. The antagonistic effect of mepazine on methadone was highly probable. The mechanical method failed to show any effect.

Methadone and Amiphenazole (Fig. 1D and 2D). Methadone and amiphenazole produced a highly probable and a significant degree of analgesia, respectively. There was no difference, however, between the effect of the drug combination and methadone. With the mechanical method, the combination had a probable effect which did not, however, differ from that of methadone. The effect of the other medications was nil.

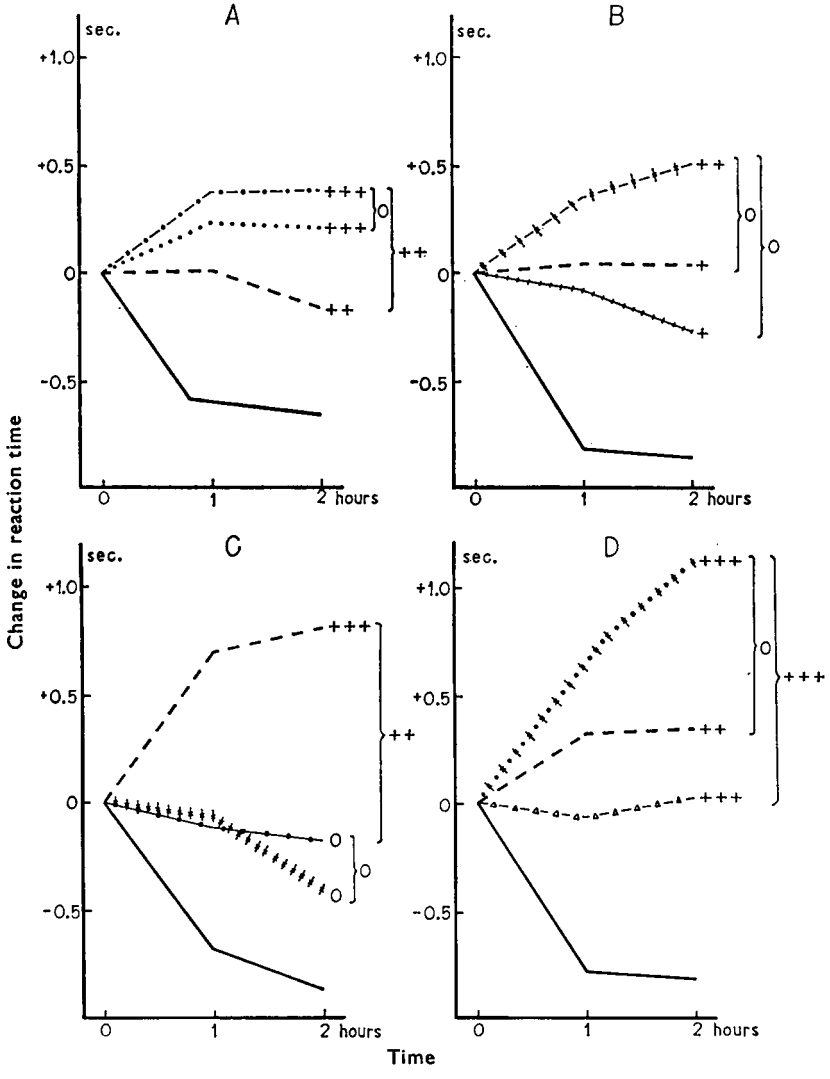

FIG. 1. Average analgesic effect tested by radiant-heat stimulation (see text for further detail).

Symbols by brackets denote statistical significance between curves. +++ = P<0·001; ++ = P 0·001 − 0·01; + = P 0·01 − 0·05; O = P>0·05.

- methadone
- chlorpromazine
- methadone+chlorpromazine
- acepromazine
- methadone+acepromazine
- mepazine
- methadone+mepazine
- amiphenazole
- methadone+amiphenazole
- placebo

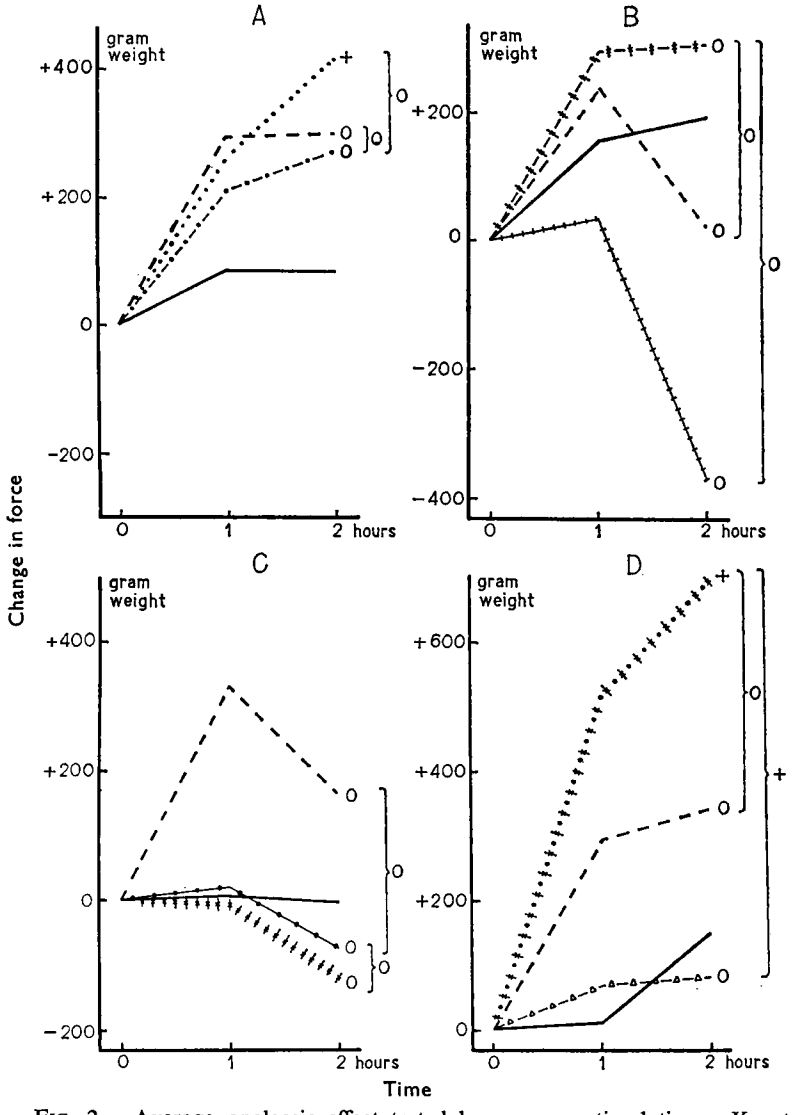

FIG. 2. Average analgesic effect tested by pressure stimulation. Key to symbols is below FIG. 1.

DISCUSSION

Studies on the analgesic action of drugs are associated with considerable methodological difficulties. Thus, the conditions encountered in experimentally induced pain and in clinical pain differ both aetiologically and psychologically. But, study of the synergism and antagonism of different drugs by means of double-blind tests in clinical pain is extremely laborious, in the large number of patients, workers and time involved. For this reason, the present study was made on experimental pain.

When using the experimental pain methods, it is important to bear in mind that different methods may give varying results with the same drug[14]. We therefore considered it reasonable to use two methods with different types of stimulus concurrently; one thermal, the other mechanical.

We showed previously[14] that untrained test subjects could be used, if 10–12 subjects were used for each drug administration. Therefore, except in the acepromazine series, each medication in the present investigation was tested in the same number of subjects.

The thermal method disclosed methadone analgesia in all experiments. The effect of methadone in the acepromazine experiments (Fig. 1B) was only probable, by reason of the small numbers in the experiment.

In the other experiments, highly probable (Fig. 1A and B) and significant (Fig. 1C) effects were obtained.

A constant finding, both in this investigation and in a previous one[14], is the fall in the placebo curves.

Of the three phenothiazine derivatives tested, two exerted an analgesic effect, one, chlorpromazine, was significant, the other, acepromazine being probable. On the other hand, mepazine lacked any analgesic action. As regards the synergistic action of these derivatives on the analgesia produced by methadone, no definite effect could be observed. Mepazine, was, on the contrary, antagonistic.

It is thus interesting to note that mepazine, which had no analgesic potency, antagonised methadone, whereas chlorpromazine and acepromazine, which had analgesic actions of their own, did not antagonise methadone.

The analgesic action of chlorpromazine has been demonstrated earlier in experimental pain, using another kind of thermal method[16], as well as in post-operative pain[7]. The synergism between phenothiazine derivatives and morphine found in previous clinical reports cannot, however, be extended to be valid for phenothiazine derivatives and methadone in experimental pain.

The phenylthiazole derivative amiphenazole has been shown not to reduce the analgesic action of morphine in clinical trials[12,13]. Our results in Figure 1D show that, in experimental pain also, amiphenazole does not antagonise methadone analgesia. This investigation also shows amiphenazole to be analgesic.

The mechanical method did not reveal any analgesic action in any instance, and seems unsuitable for this purpose. It should however, be noted that in the original description of this method[15], the test area was the skin of the forehead. In the present investigation, this area was used for the thermal method and could not be used simultaneously for both methods.

Side-effects. Of the three phenothiazine derivatives mepazine lacked side-effects, whereas chlorpromazine and acepromazine produced drowsiness, nasal congestion, orthostatic hypotension, palpitation and nausea. In combination with methadone, the incidence of hypotension and nausea was still higher. The hypotensive action of acepromazine was greater

than that of chlorpromazine, which caused us to administer smaller doses of the former.

The clinical experience of amiphenazole as an antagonist of the side-effects of morphine[11–13] is interesting, in view of our finding that amiphenazole did not reduce the side-effects of methadone.

References

1. Courvoisier, Fournel, Ducrot, Kolsky and Kretschet, *Arch. int. Pharmacodyn.*, 1953, **92**, 305.
2. Howell, Harth and Dietrich, *Practitioner*, 1954, **173**, 172.
3. Dundee, *Brit. J. Anaesth.*, 1954, **26**, 357.
4. Sandove, Levin, Rose, Schwartz and Witt, *J. Amer. med. Ass.*, 1954, **155**, 626.
5. Haeger, *Sv. Läkartidn.*, 1954, **47**, 3084.
6. Löfström, *ibid*, 1954, **47**, 2989.
7. Jackson and Smith, *Ann. intern. med.*, 1956, **45**, 640.
8. Delay, Pichot and Ropert, *Presse Médicale*, 1957, **65**, 491.
9. Nieschulz, Popendiker and Sack, *Arzneimit.-Forsch.*, 1954, **4**, 232.
10. Kopf, *Nord. Med.*, 1955, **54**, 1779.
11. Gershow, Bruce, Orchard and Shaw, *Brit. med. J.*, 1958, **2**, 366.
12. McKeogh and Shaw, *ibid.*, 1956, **1**, 142.
13. Holmes, *Lancet*, 1956, **2**, 765.
14. Boréus and Sandberg, *Acta pharm. tox. Kbh.*, 1955, **11**, 198.
15. Hardy, Wolff and Goodell, *J. appl. Physiol.*, 1952, **5**, 247.
16. Hougs and Skouby, *Acta pharm. tox. Kbh.*, 1957, **13**, 405.