THE DEVELOPMENT OF TOLERANCE TO MORPHINE IN RATS CONCURRENTLY TREATED WITH CHLORPROMAZINE*

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Using a modified hot plate method for measuring analgesia in rats, the analgesic dose of morphine was estimated to be about 5 mg./kg. This dose increased by 50 per cent the time the animals withstood exposure before reacting to the thermal stimulation. When chlorpromazine 1·5 mg./kg. was given simultaneously with morphine, a similar degree of analgesia was obtained with about 2 mg./kg. of the narcotic. The sensitivity decreased markedly in rats receiving daily injections of analgesic doses of morphine. Chlorpromazine did not retard the development of tolerance to morphine in these rats. Some of the toxic effects of morphine, however, were less marked in the rats treated with both drugs than those treated with morphine alone.

AFTER repeated administration of morphine to an animal or to man its analgesic effect decreases. This reduction is a manifestation of tolerance. The rate at which tolerance develops is related to the dose For example, Schmidt and Livingston[1] reported that, when dogs were treated daily with 30 to 60 mg./kg. of morphine, tolerance to the narcotic effect developed within one month, but when the dose was 2 to 10 mg./kg. tolerance developed only after the dogs had been treated for 15 to 20 weeks.

Since chlorpromazine has been found to potentiate the analgesic action of morphine[2,3] it has been used clinically with the narcotic, less of which is then necessary to obtain an adequate analgesic effect.[4,5] Tolerance to this combination of drugs might be expected to develop more slowly than to morphine alone, and the present investigation was initiated to ascertain whether chlorpromazine affected the development of tolerance to morphine in rats.

EXPERIMENTAL

Morphine sulphate (Merck) and chlorpromazine hydrochloride (Poulenc) were used. The weights of the drugs refer to their salts.

The animals used were male Wistar rats initially weighing 120 to 160 g.

Method

The rate at which tolerance to morphine developed was assessed during a period of seven weeks, when the rats were given single daily injections of suitable doses of the narcotic or of the combination of the narcotic and chlorpromazine. The principle is similar to that of Galysh and others[6].

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The analgesic effect was tested using a modification of Eddy and Leimbach's hot plate method[7]. The animals were placed one at a time in a large dry metal vessel fitted tightly on a water bath at 55·5°. This temperature was found to have no deleterious effect on the paws of the rats, even when they were repeatedly exposed to this stimulus either at 10-minute intervals on the same day or once daily for several weeks. The rats were found to respond to this thermal stimulation by licking the front paws or jumping out of the hot vessel. The time required to elicit either response was designated the exposure time (ET). In a preliminary test on 100 untreated rats 63 responded by licking their front paws. The mean ET for this group was $6{\cdot}63 \pm 0{\cdot}21$ seconds. The mean ET of the 37 rats which responded by jumping out of the vessel was $6{\cdot}54 \pm 0{\cdot}28$ seconds. Thus there was no significant difference in the mean ET between these two types of response. Both were therefore used as the end point in subsequent experiments.

In testing the analgesic effect of morphine, the ET of each rat in a group of 30 was first determined. Ten minutes later morphine was given by intraperitoneal injection, and the ET determined after a further 30 minutes. The per cent increases (Y) in the ET of these rats and the three logarithmic doses (X) were used in the analysis of variance and the computation of the regression equation ($Y = a + bX$). The AD50 value is the dose of morphine estimated to increase the ET by 50 per cent. It was calculated from the regression equation by substituting 50 for Y and solving for X. The variance of the AD50 was also estimated.

For comparison the changes in the ET in some experiments were converted to quantal data; an increase of 50 per cent or more was considered a positive analgesic effect. The variance of the AD50 calculated from these quantal data was considerably larger than that obtained from the graded responses, which were therefore used throughout these experiments.

The AD50 of morphine was then determined in another 30 rats, each of which was also injected intraperitoneally with chlorpromazine (1·5 mg./kg.). This dose of chlorpromazine had no analgesic action when given alone, but it potentiated the analgesic action of morphine.

Both groups of 30 rats were then given daily injections of morphine alone (Group A) at the determined AD50 of 5·09 mg./kg., or of morphine with chlorpromazine 1·5 mg./kg. (Group B) at the determined AD50 of 1·95 mg./kg. At the end of one week, the AD50 values were again determined as described above. They were found to have been increased, and so the new AD50 figures were used in the second week of dosing. This process was repeated at the end of the third and subsequent weeks for a total of seven weeks, the dose of chlorpromazine being kept constant throughout.

Since the experimental period for the rats was relatively long, it was considered desirable to ascertain the influence of age on the analgesic effect of morphine. The AD50 of morphine was therefore determined in two further groups of 30 animals at the beginning and at the end of the seven-week period, throughout which no drug was administered.

These rats were weighed weekly, and after determining the final AD50 values the animals were killed and some organs weighed.

RESULTS

The initial AD50 of morphine in the first group (A) of rats which received this analgesic alone was 5·09 mg./kg., whereas in the second group (B) which in addition had received chlorpromazine (1·5 mg./kg.) the AD50 was 1·95 mg./kg. The AD50 values obtained with these two groups during the entire experimental period are listed in Table I, from which it is evident that there is a considerable increase in both groups.

TABLE I

THE AD50 VALUES OF MORPHINE (IN MG./KG.) DETERMINED WEEKLY IN RATS RECEIVING DAILY INJECTIONS OF (A) MORPHINE OR (B) MORPHINE AND CHLORPROMAZINE (1·5 MG./KG.) DURING A PERIOD OF 7 WEEKS

	Weeks							
Group	0	1	2	3	4	5	6	7
A	5·09	6·62	10·26	9·27	13·06	19·10	20·61	24·72
B	1·95	2·96	2·74	2·47	9·27	8·10	9·27	9·44

To estimate the rate of tolerance development in these two groups with more precision, regression lines were computed from the logarithmic AD50 values and the time in weeks at which they were determined. The slope (b) of the regression line and the variance (s_b) are respectively for Group A, 0·093 and 0·0016 and for Group B, 0·094 and 0·0070. Tolerance to morphine developed at a similar rate in these groups. In calculating the regression line the AD50 values were weighted with the reciprocals of their variance. This procedure theoretically improves the accuracy of the estimated slope. It also reduces the variance of this estimate as the s_b was 0·0016 when the AD50 values were weighted, whereas from the unweighted values it was 0·0129. The AD50 values determined in the third and fourth weeks in Group B were inaccurate as their variances were large. However, in spite of these marked deviations the regression of AD50 plotted against time was highly significant.

TABLE II

THE INITIAL AND FINAL AD50 VALUES ± S.E. (MG./KG.) OF MORPHINE IN RATS RECEIVING DAILY INJECTIONS OF (A) MORPHINE, (B) MORPHINE AND CHLORPROMAZINE, AND IN RATS GIVEN (C) MORPHINE AND (D) MORPHINE AND CHLORPROMAZINE AT THE BEGINNING AND END OF THE EXPERIMENTAL PERIOD ONLY

Group	Initial	Final
A	5·09 ± 0·92	24·72 ± 2·31
B	1·95 ± 0·19	9·44 ± 0·94
C	4·70 ± 1·12	6·30 ± 0·51
D	2·16 ± 0·22	2·97 ± 0·32

In Table II are listed the initial and final AD50 values of morphine in the two groups (C and D) of rats which received no daily treatment.

There was a slight but not significant increase in the AD50 values at the end of this period. This is in sharp contrast to the marked increases observed in the Groups A and B, the initial and final values of which are also listed in this Table for comparison.

Although the combination of chlorpromazine and morphine was not superior to morphine alone as far as development of tolerance is concerned, it appeared to be less toxic; for example, the growth was slightly but significantly more retarded in the rats treated with morphine (Group A) than those treated with the combination (Group B). This is shown in Table III. Alopecia areata was observed in all the animals during the

TABLE III

THE BODY WEIGHT IN G. (MEAN ± S.E.) OF THE FOUR GROUPS OF RATS (GROUP DESIGNATIONS AS IN TABLE II)

Group	Initial	Final
A	139 ± 2·9	231 ± 4·8
B	138 ± 4·1	249 ± 4·7
C	138 ± 1·6	258 ± 7·9
D	138 ± 2·9	268 ± 7·2

latter half of the experimental period, but the rats in Group A were affected to a greater extent than those in Group B. From the weights of some of the organs (see Table IV), it may be noted that the increase in

TABLE IV

THE WEIGHT (MEAN ± S.E.) OF ADRENALS, THYMUS AND THYROID IN MG./G. BODY WEIGHT OF RATS TREATED WITH (A) MORPHINE, (B) MORPHINE AND CHLORPROMAZINE AND (C) NEITHER

Group	Adrenals	Thymus	Thyroid
A	0·137 ± 0·003	1·17 ± 0·07	0·051 ± 0·001
B	0·133 ± 0·002	1·34 ± 0·06	0·046 ± 0·002
C	0·126 ± 0·002	1·70 ± 0·06	0·053 ± 0·002

the weight of the adrenals and the decrease in the weight of the thymus were more marked in Group A than in Group B. However, the weight of the thyroid was reduced in Group B but not in Group A.

DISCUSSION

A potentiation of the analgesic action of morphine by chlorpromazine has been observed in rats. The AD50 of morphine was reduced by three-fifths when a relatively small dose of chlorpromazine (1·5 mg./kg.) was given simultaneously. The magnitude of potentiation was similar to that reported by others; for example, Wirth[3] found that the analgesic dose of morphine was reduced by a half or two-thirds in the presence of chlorpromazine. According to the data presented by Courvoisier and

others[2], the AD50 of morphine was about 25 mg./kg. when it was given alone. However, when the rats had recieved chlorpromazine (5 mg./kg.), the AD50 of morphine was about 6 mg./kg. The greater potentiation was evidently related to the larger dose of chlorpromazine.

Although the rats in Groups C and D were less sensitive to morphine at the end of the 7 weeks than at the beginning, the increase in the AD50 over the period was very small (Table II). The increase in the AD50 values in the rats in Groups A and B was therefore a result of tolerance to the drug administered.

The rate at which tolerance to morphine developed in the rats was slightly slower than that observed by Galysh and colleagues[6], and the coefficient of variance was smaller. This is one of the advantages of using weighted AD50 values in calculating the regression line.

The present results show that tolerance to morphine developed at the same rate in rats treated with morphine and chlorpromazine (in spite of much smaller doses of the narcotic), as in those treated with morphine alone. The mechanism underlying this unexpected finding is not evident. It is not likely to be a result of a tolerance to chlorpromazine, since we found that the potentiating effect of this drug was identical in two other groups of rats: one group had received chlorpromazine daily for 7 weeks and the other had received saline.

There was a significant reduction in the increase in body weight in rats treated with morphine. Loss of hair, an increase in the weight of adrenals and a decrease in the weight of the thymus were also noted. These effects of morphine have been reported by others[8–10]. However, it is of interest to note that these changes were less marked in rats treated with morphine and chlorpromazine. Since the changes may be considered as signs of toxicity, the combination of both drugs was less toxic than morphine alone.

Another difference between the rats treated with morphine and those treated with the combination was the effects on spontaneous motor activity. The injection of morphine normally induced sedation. After 3 to 4 weeks' daily treatment this narcotic elicited excitation instead of sedation. This stimulation of the central nervous system after morphine injection in morphine-tolerant rats has been observed by others[9,11]. Excitation, however, was not seen in the rats treated with morphine and chlorpromazine.

The weight of the thyroid was less in rats treated with morphine and chlorpromazine than in those treated with morphine alone. This effect was therefore likely to be related to chlorpromazine, which has been reported to impede the uptake of ^{131}I by the thyroid in guinea pigs[12] and to prevent the histological changes in the thyroid of rats induced by exposure to cold[13].

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