ANALGESIC ACTION OF CHLORPROMAZINE AND RESERPINE IN RELATION TO THAT OF MORPHINE

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An analgesic action of reserpine is reported in mice. This effect, detected by the hot-plate method, is of little, or only of moderate intensity, in the first 2 hr. after injection; maximal intensity is obtained 48–72 hr. after the injection of the drug, and then decreases gradually; after 144 hr. injected animals behave again like controls. The analgesic effect of reserpine seems to differ from that shown by morphine or that shown by chlorpromazine which is quicker in onset and disappears in a few hours. Reserpine potentiates the analgesic action of morphine. This potentiation can be observed 2 hr. after the injection of reserpine. The early potentiating period is followed by a later period of summation of effects. Since a short interval after the injection, reserpine does not exert any analgesic effect, but is able to potentiate morphine analgesia, this action has been regarded as a direct one; the later period of analgesia and summation of effects with morphine has been interpreted as an indirect action of the drug.

THE importance of the study of analgesic actions of tranquillising drugs has been emphasised in a recent symposium at the New York Academy of Sciences (Annals, 1960). Of special interest are the relations existing between the analgesic actions of tranquillising drugs and those of analgesics of the morphine group, in view of the possibility that a better knowledge of the mechanism of action of either group may be attained. On the other hand, the measurement of such a complex phenomenon as pain, by the common algesimetric methods, might indicate differences in the mode of action of drugs, some exerting their effect through a central action, as those of the morphine group, and others by a local action, interfering in the intimate mechanism of production of the painful stimuli in the skin or in the peripheral nervous endings.

Recent experiments indicate the presence in the body of pain producing factors like acetylcholine, histamine, 5-hydroxytryptamine, (5-HT) and polypeptides (Armstrong, Jepson, Keele, and Stewart, 1957), the release of which might contribute to the stimulation of the peripheral nervous endings and therefore to the genesis of painful stimuli. Heating, especially, has been shown to release certain of these factors (bradykinin), at temperatures as low as 45°. Reserpine, but not chlorpromazine, appears to interfere with the skin reaction at that temperature (Rocha e Silva and Antonio, 1960). At 55° histamine and 5-HT might also be released and participate in the reaction.

It seems established that chlorpromazine, besides having an analgesic action of its own, exerts a potentiating action on morphine, when both drugs are simultaneously injected in laboratory animals, as has been described by Courvoisier, Fournel, Ducrot, Kolsky and Koetschet (1953),

Schneider (1954), Frommel and Fleury (1959), though Kopera and Armitage (1954) were unable to demonstrate such an action using 1·5 mg./kg. morphine sulphate and 10 mg./kg. chlorpromazine.

The analgesic action of reserpine has not been admitted (Schneider, 1954; Bein, 1953; Bein, Gross, Tripod and Meier, 1953; Bein, 1956). An antagonism between reserpine and the analgesic action of morphine has been described (Schneider, 1954); on the other hand Tripod and Gross (1957) observed the analgesic action of morphine to be potentiated by reserpine in mice.

A comparative and detailed study of the analgesic actions of these groups of drugs as done in the foregoing paper, might contribute to a clarification of some still obscure points.

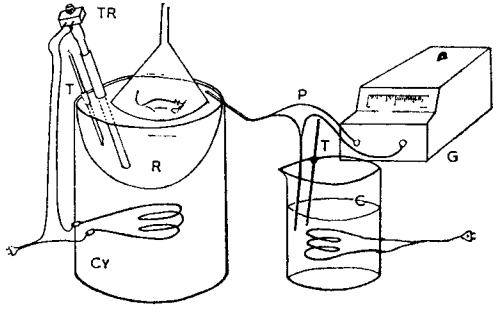

FIG. 1. Apparatus used—Cy: cylinder; R: copper receiver; TR: thermo-regulator; T: thermometers; P: thermocouple; C: cylinder; G: galvanometer.

MATERIAL AND METHODS

The experiments were carried out on male mice weighing between 10 and 20 g.

The analgesic action was measured by the hot-plate method. The apparatus (Fig. 1) consisted of a metal cylinder to which a copper semi-spherical receiver filled with water was adapted. The system was closed with a metal plate of 20 cm. diameter, on which the animals were placed. The temperature is adjusted by a thermo-regulator which corrected wide variations of temperature. A finer regulation was obtained using a thermo-couple. One of the poles of the couple was fixed to the plate by an adhesive strip and the other immersed in a cylinder of water, gradually heated by an electric resistance. The thermocouple was connected to the poles of a galvanometer. The temperature on the plate was accurately determined as being equal to that of the water bath when the galvanometer read zero.

The reaction time (RT) was measured from the moment the animal was placed on the plate until it presented any sign of discomfort as licking of both front paws simultaneously or rubbing the nose with both paws. Sometimes, the end point was indicated by a sudden jump.

The animal was restrained under a glass funnel.

The reaction time at 45° for a group of 20 mice, each used only once had a mean ± its standard error of 65·5 ± 7·1 sec.; at 50° for 50 mice it was 13·5 ± 0·5; at 55° for 75 mice it was 5·5 ± 0·2 and at 60° for 75 mice 5·7 ± 0·2 (Fig. 2). We have selected 55° for all assays since the error arising from small variations of temperature is small.

Drugs used. Reserpine: Serpasol Ciba (crystallised pure alkaloid of rauwolfia), injectable (2·5 mg./ml.). Chlorpromazine: Amplictil Rhodia (chloro-3-dimethylamine-3-propyl-10-phenothiazine chlorhydrate), injectable (25 mg./ml.). Morphine: morphine hydrochloride. Enila S.A.

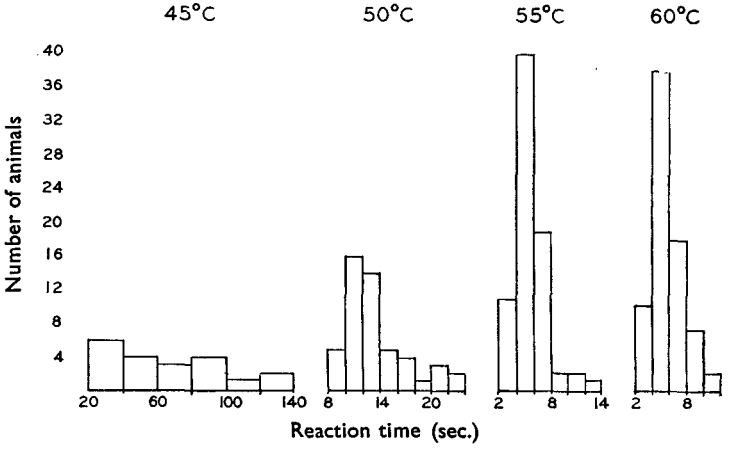

FIG. 2. Distribution of Normal Animals at 45, 50, 55, and 60° C. (temperatures of the plate).

Animals were injected subcutaneously and solutions were prepared in such concentrations that each animal received a maximum of 1 ml. Dilutions were made in distilled water.

Mice treated with reserpine received a single dose of the drug and the RT was measured 2, 24, 48, 72, 96, 120 and 144 hr. after the injection. Animals treated with chlorpromazine received a single dose of the drug and the RT was measured 1, 2, 4 and 24 hr. after the injection. Animals treated with reserpine and morphine received a single dose of reserpine 2 hr. before and one of morphine 30 min. before the measurement of the RT; 24 and 48 hr. after the injection of reserpine 2 further doses of morphine were given and 30 min. thereafter the RT was again measured.

Controls for this group received one dose of morphine daily, 30 min. before the determinations of the RT.

A second control group did not receive any treatment and the RT was measured in a sequence similar to that for the specified groups. That is, in the controls for the group treated with reserpine, the RT measurements were done at zero hour and at 24, 48, 72, 96, 120 and 144 hr. In the controls for the group treated with chlorpromazine the RT measurements were done at zero hour and 1, 4 and 24 hr. as with the animals submitted to the drug.

RESULTS

Effect of Chlorpromazine on RT *at* 55°

A group of 50 mice treated with 5 mg./kg. of chlorpromazine (0·1 mg./ml.) show an increase in RT (Table I, Fig. 3), 1 hr. after the injection of the drug and this is maximal around 2 hr. No significant difference

TABLE I

REACTION TIME (RT) VALUES OF A GROUP OF ANIMALS INJECTED WITH 5 MG./KG. CHLORPROMAZINE. COMPARISON WITH VALUES OF CONTROL GROUP

Dose	RT (Mean ± SE) at 55°				Number of animals
	1 hr. after inj.	2 hr. after inj.	4 hr. after inj.	24 hr. after inj.	
5 mg./kg.	49·6 ± 7·1	90·7 ± 6·0	71·9 ± 7·3	9·8 ± 0·5	50
Controls	6·3 ± 0·3	9·6 ± 0·7	9·6 ± 0·5	9·9 ± 0·5	30

is seen between treated (50) and control animals (30) 24 hr. after the drug has been given.

There were no deaths.

Effect of Reserpine on RT *at* 55°

Animals (100) injected with 5 mg./kg. reserpine (0·1 mg./ml.) presented a gradual increase in RT (Table II) with a maximum around 48 hr. after the injection. The RT values decreased thereafter falling to those of the controls (50 mice) around 144 hr. after administration. Of the 100 test

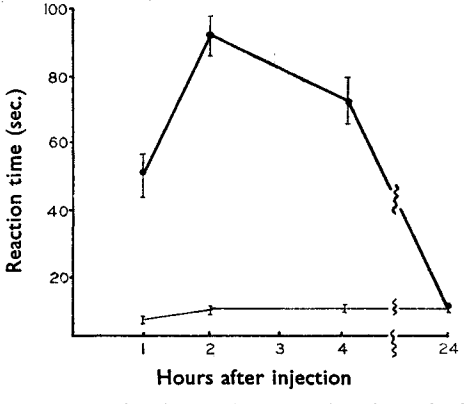

FIG. 3. Effect of chlorpromazine (●—●) on reaction time of mice submitted to the hot plate test (55° C.). Comparison with a control group (—).

animals, 24 died: 1 between 24 and 48 hr. after the administration of the drug, 8 between 48 and 72 hr., 11 between 72 and 96 hr., 3 between 96 and 120 hr., 1 between 120 and 144 hr.

Animals (50) treated with 1 mg./kg. reserpine (0·02 mg./ml.), were distributed in a similar curve with lower mean RT values. Maximum RT

in this group was observed around 72 hr. after the injection of the drug. No difference from the control group (50 mice) is seen 120 hr. after the drug (Table II). No deaths were observed in this group.

TABLE II

REACTION TIME (RT) VALUES OF A GROUP OF ANIMALS INJECTED WITH 1 AND 5 MG./KG. RESERPINE, COMPARED WITH A CONTROL GROUP

Dose	RT (Mean ± SE) at 55°							Number of animals
	2 hr. after inj.	24 hr. after inj.	48 hr. after inj.	72 hr. after inj.	96 hr. after inj.	120 hr. after inj.	144 hr. after inj.	
1 mg./kg.	5·6 ± 0·4	10·2 ± 0·6	14·7 ± 0·7	15·5 ± 0·8	13·2 ± 0·8	8·4 ± 0·4	8·2 ± 0·4	50
5 mg./kg.	9·9 ± 1·9	21·0 ± 1·5	28·7 ± 1·8	23·9 ± 1·7	17·7 ± 0·8	14·1 ± 0·7	10·4 ± 0·6	100
Controls	5·8 ± 0·3	11·3 ± 0·7	10·6 ± 0·5	11·1 ± 0·4	11·0 ± 0·5	9·8 ± 0·4	8·6 ± 0·4	50

Effect of Reserpine and Morphine on RT *at* 55°

The combined effects of reserpine and morphine were observed in two assays. In the first, threshold doses of 1 mg./kg. (0·02 mg./ml.) of both substances were injected 2 hr. and 30 min. before the measurement of the RT. Those values were compared with control animals separately injected with each of the two drugs.

In the group of 20 animals which received 1 mg./kg. morphine only, an increase in the RT from 5·5 ± 0·2 sec. (normal values) to 7·8 ± 0·4 sec.

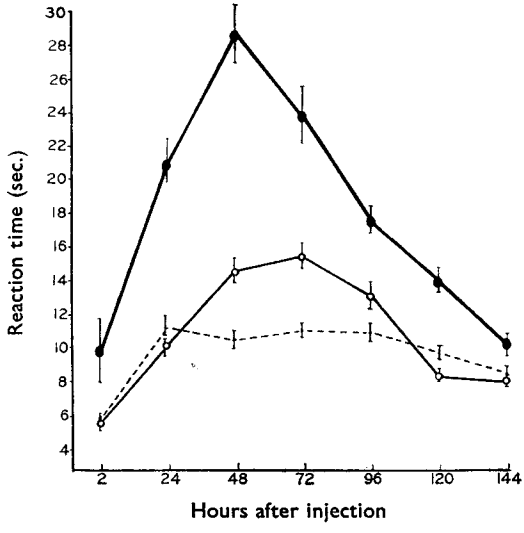

FIG. 4. Effect of reserpine (●—●, 5 mg.; ○—○, 1 mg./kg.) on reaction time of mice submitted to the hot plate test (55° C.), compared with a control group (- - -).

was observed. In the group of 50 animals which received 1 mg./kg. reserpine only, the increase in the RT was not significant. In the group which received both drugs (40 mice) a significant increase in the RT was seen, to 14·1 ± 1·0 sec.

It seems clear that a potentiation of the analgesic effect of morphine has occurred. Table III.

TABLE III

REACTION TIME (RT) VALUES OF ANIMALS TREATED WITH THRESHOLD DOSES OF RESERPINE AND MORPHINE

Treatment	RT (Mean ± SE)	Number of animals
Reserpine (1 mg./kg.) + Morphine (1 mg./kg.)	14·1 ± 1·0	40
Reserpine (1 mg./kg.)*	5·6 ± 0·4	50
Morphine (1 mg./kg.)	7·8 ± 0·4	20

*Data from Table II.

TABLE IV

REACTION TIME (RT) VALUES IN THE GROUP SUBMITTED TO COMBINED TREATMENT: RESERPINE + MORPHINE. COMPARISON WITH THE GROUP SUBMITTED ONLY TO THE ACTION OF RESERPINE AND TO THE GROUP SUBMITTED ONLY TO THE ACTION OF MORPHINE

Treatment	RT (Mean ± SE) at 55°			Number of animals
	2 hr. after reserpine inj. 30 min. after 1st dose of morphine	24 hr. after reserpine inj. 30 min. after 2nd dose of morphine	48 hr. after reserpine inj. 30 min. after 3rd dose of morphine	
Reserpine 5 mg./kg. (single dose) + Morphine 5 mg./kg. (3 doses)	67·3 ± 3·6	44·5 ± 3·5	54·4 ± 3·6	100
Reserpine* 5 mg./kg. (single dose)	2 hr. after reserpine inj.	24 hr. after reserpine inj.	48 hr. after reserpine inj.	
	9·9 ± 1·9	21·0 ± 1·5	28·7 ± 1·8	100
Morphine 5 mg./kg. (3 doses)	30 min. after 1st dose	30 min. after 2nd dose	30 min. after 3rd dose	
	22·5 ± 2·1	21·5 ± 1·7	21·4 ± 1·9	45

*Data from Table II.

In the second assay both drugs were injected in a dose of 5 mg./kg., reserpine 2 hr. and morphine, 30 min. before the assay.

In a group of 100 animals, new determinations of RT were made 24 and 48 hr. after the injection of reserpine and 30 min. after a new dose of morphine. The results can be seen in Table IV and Figs. 5 and 6.

In the animals which received only morphine (45 animals) 30 min. before the assay and whose RT were determined three times at intervals of 24 hr., the values were 22·5 ± 2·1; 21·5 ± 1·7 and 21·4 ± 1·9 respectively (Table IV).

The 100 animals which received only reserpine showed a small increase (9·9 ± 1·9 sec.) in their RT compared with normal values, 2 hr. after and a remarkable increase 24 and 48 hr. after the injection of the drug, respectively to 21·0 ± 1·5 and 28·7 ± 1·8 sec. (Table IV).

Of interest is the fact that animals which received both drugs in the first day presented a remarkable increase in the RT to 67·3 ± 3·6 sec., which might indicate a clear potentiating effect of reserpine towards

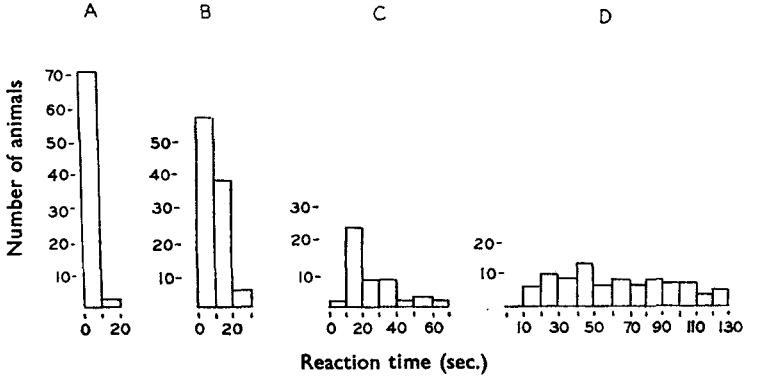

FIG. 5. Distribution of animals submitted to the combined treatment reserpine + morphine (2 hr. after reserpine and 30 min. after 1st dose of morphine). Comparison with reserpine treated, morphine treated and control animals.

A: controls (no treatment)—75 animals.

B: 2 hr. after reserpine (5 mg./kg.)—100 mice.

C: 30 min. after 1st dose of morphine (5 mg./kg.)—45 animals.

D: combined treatment: 2 hr. after reserpine (5 mg./kg.) and 30 min. after 1st dose of morphine (5 mg./kg.)—100 mice (10 mice over 130 sec.).

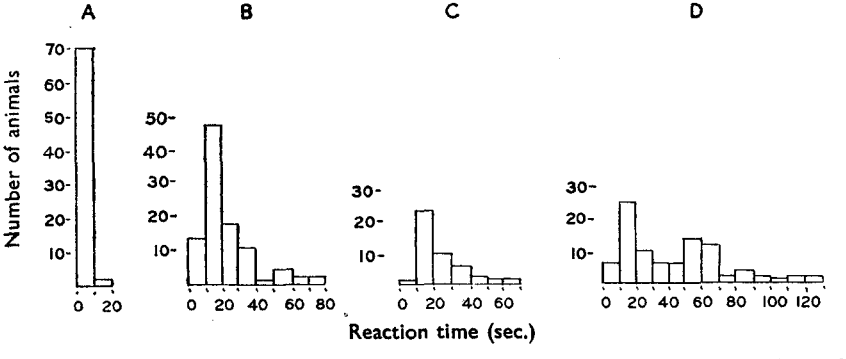

FIG. 6. The same as Fig. 5, 24 hr. after reserpine and 30 min. after 2nd dose of morphine.

A: controls (no treatment)—75 mice.

B: 24 hr. after reserpine (5 mg./kg.)—100 mice.

C: 30 min. after 2nd dose of morphine (5 mg./kg.)—45 mice.

D: combined treatment: 24 hr. after reserpine (5 mg./kg.) and 30 min. after 2nd dose of morphine (5 mg./kg.)—100 mice (5 mice for 130 sec.).

morphine. In subsequent days also, an increase in the RT was observed but this more as if a summation of the effects of reserpine and morphine had occurred, since both produce an increase in the RT as seen in Table IV.

In the period between 2 and 24 hr. after reserpine, 3 animals died in this group.

Conclusions and Discussion

Our results suggest that in mice there is an analgesic action due to reserpine, that can be clearly detected after 24 hr. and which remains for some days.

In the first 2 hr. after the injection of reserpine the effect is still undetectable or of moderate intensity; the maximum effect is obtained in 48–72 hr. and then decreases gradually. After 144 hr. the effect disappears and the injected animals behave like the controls.

The analgesic effect of reserpine seems to differ from that of morphine or chlorpromazine. That of chlorpromazine is of quick onset and is transient.

Between 48 and 72 hr. after the injection of reserpine the analgesic effect is more or as intense as that observed 30 min. after the injection of an equivalent dose of morphine, but less intense than that observed in the first 4 hr. after the injection of a similar dose of chlorpromazine.

Reserpine potentiates the action of morphine. This potentiation can be observed 2 hr. after the injection of reserpine. The early potentiating period is followed by a later period of summation of effects. When morphine is injected 24 or 48 hr. after reserpine, the intensity of the analgesic effect is less than when administered only 2 hr. after reserpine.

The initial potentiating effect can be clearly seen when threshold doses of reserpine and morphine are used.

Comparing the analgesic action of tranquillisers and their effects upon morphine analgesia, it seems apparent that a striking difference can be observed between the two classes of drugs, the one represented by chlorpromazine and the other by reserpine. Chlorpromazine has a definite and early analgesic activity, which disappears after a few hours; reserpine on the other hand shows an analgesic action only 24 or 48 hr. after the injection.

When tested in combination with morphine, both drugs enhance its effects. Reserpine, however, in the first hours after the injection, definitely potentiates the analgesia induced by morphine; the later summation of effects of reserpine and morphine, appearing 24 hr. after the injection of the rauwolfia alkaloid, has no parallel with chlorpromazine since the effect of chlorpromazine is not detectable after a few hours.

Our experiments are more concerned with the relation between reserpine and morphine and with the differences seen between a short or a longer interval after the injection of reserpine. In the early period a true potentiation is observed, since reserpine by itself has no analgesic action or only a moderate one, but after 24 or 48 hr., a definite increase in RT is observed. When morphine is then injected, a simple summation of the analgesic effects of reserpine and morphine occurs.

One might speculate on the mechanism of this dual effect of reserpine when combined with morphine. As the analgesic action of reserpine is observed only a long interval after the injection of the drug and remains for some days it could be interpreted as an indirect action, since after 48 hr., only traces of it are detectable in the brain (Plummer, Sheppard, and Schulert, 1957; Sheppard, Hui, Plummer, Peets and Giletti, 1958). This action could involve a participation of catechol and indole amines, since there is some indication that the time required for the injected animal to behave like the controls is the time necessary to restore those amines in the nervous structures of the brain after depletion by reserpine. But note that these last experiments were carried out on rabbits (Pletscher, Shore and Brodie, 1956). A short interval after injection, reserpine does not exert any analgesic effect, but is able to potentiate morphine analgesia. This could be interpreted as a direct action of the drug, if we assume that at this stage the highest concentrations of reserpine would be present in the brain.

Reserpine by itself is able to potentiate the analgesic action of morphine, but its analgesic effect would be an indirect one, probably following depletion of catechol or indole amines.

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