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CONDITIONS INFLUENCING THE INHIBITION, BY ANALGESIC DRUGS, OF THE RESPONSE TO INTRAPERITONEAL INJECTIONS OF PHENYLBENZOQUINONE IN MICE

BY

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Vander Wende & Margolin (1956) reported that intraperitoneal injections of iodinated contrast agents elicited a characteristic stretching response in rats which could be abolished by narcotic analgesics. They put forward evidence to show that the activity measured for the narcotics was an analgesic one since estimates of potency obtained in this assay paralleled those obtained by other methods. Their test, however, was not sufficiently sensitive to detect weak analgesic activity. With the introduction of 2-phenyl-1,4-benzoquinone as a nociceptive agent in mice (Siegmund, Cadmus & Go Lu, 1957a), both morphine-like and acetylsalicylic acid-like analgesic activity could be measured (Siegmund, Cadmus & Go Lu, 1957b; Eckhardt, Cheplovitz, Lipo & Govier, 1958; Carroll & Lim, 1958; Hendershot & Forsaith, 1959).

Earlier workers treated the incidence of stretching as a quantal response but more recently the treatment of the response as a graded one has improved the precision of the assay (Hendershot & Forsaith, 1959).

The following account describes further modification of conditions of the test so that estimates of potency for both strong and weak analgesics may be made with equal precision using similar numbers of animals in a graded assay. The authors have described the method in part to the joint meeting of the British and Scandinavian Pharmacological Societies in Copenhagen, 1960.

METHODS

Preparation of phenylbenzoquinone solution. Phenylbenzoquinone has a low solubility in water and is not stable in aqueous solution. The solubility may be increased by the inclusion of 5% alcohol (Siegmund *et al.*, 1957a) and the stability increased by protection from light. This solution is best prepared immediately before use by dissolving phenylbenzoquinone in warm absolute alcohol (up to 4 mg/ml.), and diluting with water at 40° C. Such solutions, maintained at 34° C and protected from light retain their potency for at least 10 hr.

Animals. L.A.C. grey male mice were used throughout, 7 to 10 days after weaning, that is approximately 5 to 6 weeks old, and weighing 14 to 23 g. The experiments were planned with a random distribution among groups, with respect to days after weaning, as younger mice appear to respond more readily.

Apparatus. After injection with phenylbenzoquinone, mice were observed in a thermostatically controlled water-bath containing six mouse boxes (7 × 5 × 5 in.). The temperature of the water could be varied between 21 and 34° C and controlled to ±0.05° C. A hand tally counter was used to record the total number of stretching movements for each group of five mice in a 5-min period.

RESULTS

The stretching response to phenylbenzoquinone at room temperature

Injections of 1, 2 and 4 mg/kg of phenylbenzoquinone, intraperitoneally, caused five out of seven mice to respond within 10 min at room temperature (21° C). Groups of five reacting mice were counted over successive 5-min periods. Between 10 to 15 min after injection, the rate of responding for each of the doses had reached a maximum but was followed by a rapid exponential decline in the number of stretching movements with time, so that by 65 min after injection the responses to all three doses had reached a common value (Fig. 1).

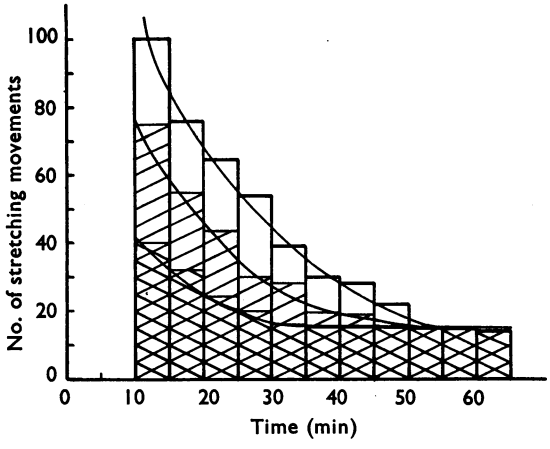

Fig. 1. Number of stretching movements occurring in groups of five mice in successive 5-min periods after intraperitoneal injection of phenylbenzoquinone. Open area, 4 mg/kg; hatched area, 2 mg/kg; cross-hatched area, 1 mg/kg.

Over limited periods a linear relationship holds between the incidence of stretching for reacting mice and the dose of phenylbenzoquinone (Fig. 2). The steepest relation between dose and rate of response occurred during the 10- to 15-min period. Moreover, the value for s/b calculated for regression during this period was shown to be minimal (Table 1).

Thus, provided that dose/effect curves for the agent after treatment with an antagonist are parallel to those for the agent alone, the best assay of analgesic potency will be obtained when counting is limited to 10 to 15 min after phenylbenzoquinone. This interval was used in the experiments described below, except where stated otherwise, and the mice were killed immediately after counting.

Assessment of analgesics at room temperature

Codeine hydrochloride. Codeine hydrochloride given by mouth inhibited the number of stretching movements in response to a fixed dose of phenylbenzoquinone in a quantitative

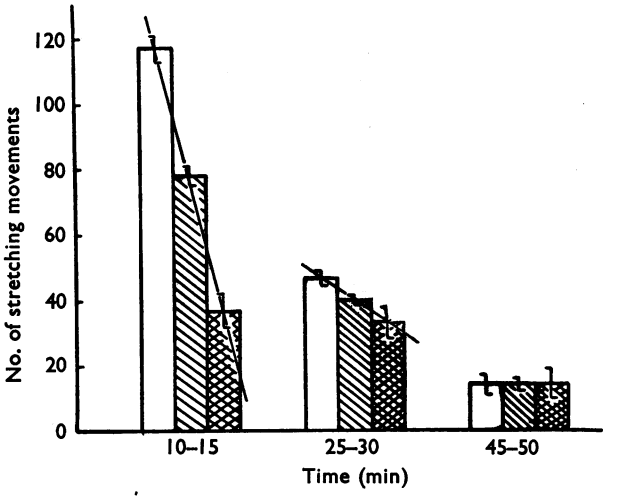

Fig. 2. Relationship between dose and number of stretching movements occurring in groups of five mice during three 5-min periods after intraperitoneal injection of phenylbenzoquinone. Open area, 4 mg/kg; hatched area, 2 mg/kg; cross-hatched area, 1 mg/kg. Standard errors are shown by the vertical lines.

TABLE 1

RELATION OF RESPONSE TO DOSE OF PHENYLBENZOQUINONE

Groups of five mice were counted in successive 5-min periods after injection. Values of b are given with fiducial limits ($P=0{\cdot}05$)

Period	b	s/b
10–15 min	129·9±17·4	0·07
25–30 min	21·7±12·7	0·29
45–50 min	8·4± 8·6	0·51

manner. A linear relationship held between the reduction in control response and the log of the dose of codeine (Fig. 3), when stretching was initiated by a standard dose of 4 mg/kg of phenylbenzoquinone, codeine having been given orally 20 min earlier. The assay was readily reproducible and analysis of results shows that regression was highly significant ($P<0.001$) without significant deviation from linearity ($P>0.05$). Codeine hydrochloride was effective over the range of doses 3 to 14 mg/kg orally, 50% protection being given by 5 mg/kg.

Acetylsalicylic acid. Although acetylsalicylic acid given by mouth reduced the incidence of stretching after phenylbenzoquinone, dose/response relationships were difficult to establish unless a large number of animals was used. The slope of the log dose/response line was very shallow (Fig. 4), the range of effective doses being very great. 50% protection against phenylquinone was given by 20 mg/kg of acetylsalicylic acid and from these results it appears that as little as 2 mg/kg orally could give significant protection. Moreover, at this level of effect, acetylsalicylic acid was as active as codeine. Dose/response lines for salicylamide and related compounds had slopes parallel to that of acetylsalicylic acid, but significantly different from codeine, Screening of such compounds for weak analgesic activity was difficult because of the large value of s/b, with the consequence that the fiducial

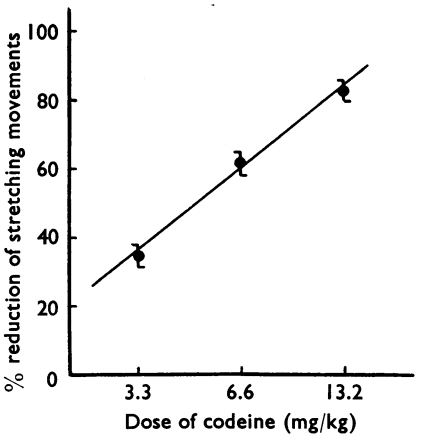

Fig. 3. The effect of codeine hydrochloride on the number of stretching movements occurring in groups of five mice, between 10 and 15 min after intraperitoneal injection of 4 mg/kg of phenylbenzoquinone, codeine being given orally 20 min before. Room temperature was 21° C. b = 81.4±11.6 (95% fiducial limits). Standard errors of the means are shown by the vertical lines.

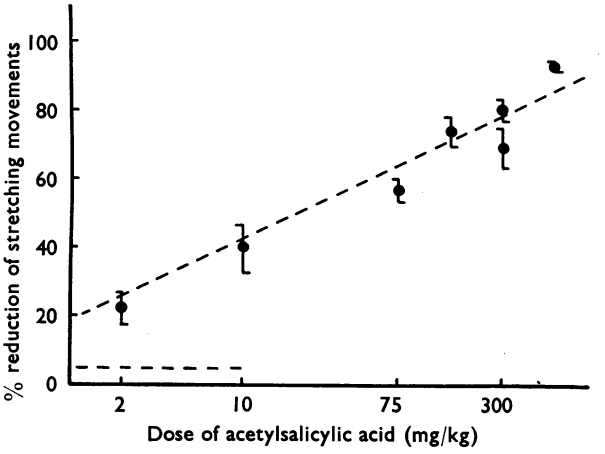

Fig. 4. The effect of acetylsalicylic acid on the number of stretching movements occurring in groups of five mice, between 10 and 15 min after intraperitoneal injection of 4 mg/kg of phenylbenzoquinone, acetylsalicylic acid being given orally 20 min before. Room temperature was 21° C. b = 23.9±3.9 (95% fiducial limits). Standard errors of the means are shown by the vertical lines. The broken line represents the standard error of control values.

limits for 50% protective doses were wide. Table 2 shows that, under these conditions of assay, weak analgesic potency cannot be estimated with precision equal to that of the narcotic analgesics. Hence, modification of the assay was sought so that weak analgesics could be estimated with equal precision, commencing with an investigation of the effect of changes in temperature.

TABLE 2

COMPARISON OF SLOPES OF LOG DOSE/RESPONSE LINES FOR STRONG AND WEAK ANALGESICS ESTIMATED AT 21° C IN THE PHENYLQUINONE ASSAY

Values of b are given with fiducial limits (P=0·05). * Regression not significant at subhypnotic doses

Drug	No. of mice	b	s/b
Morphine sulphate	120	82·76±12·02	0·07
Codeine hydrochloride	120	81·4 ±11·6	0·07
Acetylsalicylic acid	240	27·62±13·72	0·32
Salicylamide	240	*	>1·0

Variation of the conditions of assay

Temperature. Injection of phenylbenzoquinone intraperitoneally was shown to cause a reduction in body temperature, in addition to the stretching response. When the mice were kept at 34° C the body temperature no longer fell and the response to the agent increased (Fig. 5). Moreover, the proportion of mice responding after injection was now increased and it was possible to inject only five mice per group with phenylquinone and ensure that all would respond. A smaller dose of phenylquinone now caused a rate of response suitable for assessing analgesic activity at the higher temperature.

Narcotic analgesics showed very similar dose/response relationships at 34° C to those determined at 21° C. Fig. 6 shows the results of a comparison of morphine and codeine subcutaneously in twenty-five groups of five mice in a 5+5 Latin square design at 34° C. The potency of morphine was 13.2-times that of codeine, with 95% fiducial limits, 10.0 to 17.4.

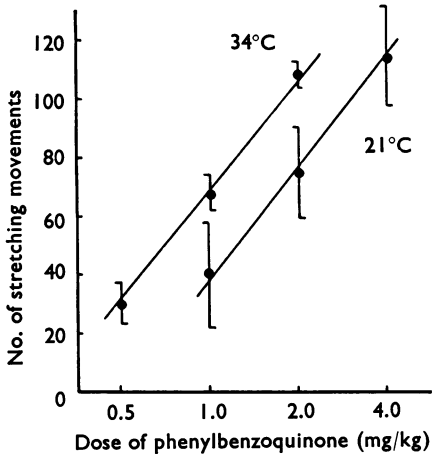

Fig. 5. The effect of external temperature on the number of stretching movements occurring in groups of five mice between 10 to 15 min after intraperitoneal injection of phenylbenzoquinone. At 21° C, each group derives from seven mice injected; at 34° C, only five mice were injected. The vertical lines show standard errors of the means.

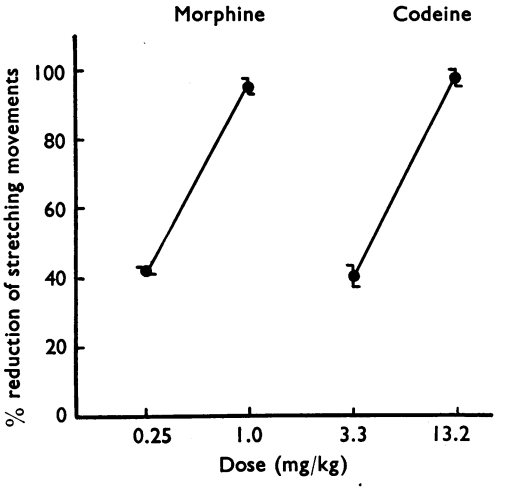

Fig. 6. Comparative assay of the activity of morphine sulphate and codeine hydrochloride in reducing the numbers of stretching movements occurring in groups of five mice between 10 and 15 min after intraperitoneal injection of 2 mg/kg of phenylbenzoquinone, drugs being injected subcutaneously 20 min before. The external temperature was 34° C.

Diet. Estimations of the activity of acetylsalicylic acid at 34° C did not show an increased precision of the assay over that at 21° C. Further modification of the assay was therefore sought by restricting the diet and by fasting the mice before use. Mice were fed on wholemeal bread and milk for 7 days and fasted overnight before use. The sensitivity to phenylbenzoquinone remained the same as in fed animals, but the onset of stretching was more rapid, and reached its maximum rate 5 to 10 min after injection of the agent. Counting was, therefore, limited to 5 to 10 min in the following assays.

Assessment of analgesics in fasted mice at elevated temperature

The dose/response relationships for narcotic analgesics remained unchanged under the new conditions. However, following dieting and fasting, the dose/response relations for acetylsalicylic acid were greatly improved. The slope was steeper and the value for s/b was reduced. Moreover, the analgesic could be shown to exert its full effect against stretching when given orally 5 min before treatment with the phenylquinone. Fig. 7 shows relationships obtained with acetylsalicylic acid, salicylamide and phenacetin; values for 50% protection were 140, 126.6 and 141.6 mg/kg respectively.

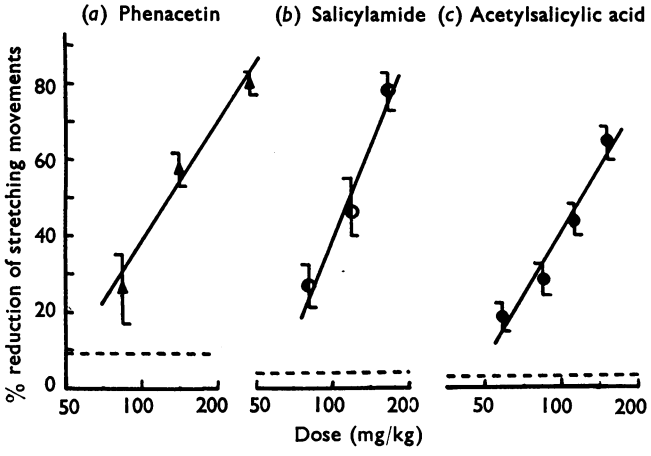

Fig. 7. Relationship between dose of analgesic and number of stretching movements occurring in groups of five mice between 5 and 10 min after intraperitoneal injection of 2 mg/kg of phenylbenzoquinone. Mice were dieted and fasted overnight before use; the analgesic was given orally 5 min before phenylbenzoquinone; the temperature was 34° C. (*a*) Phenacetin: b = 79.8±12.3; dose for 50% protection = 141.6 mg/kg (95% confidence limits, 68–294.4 mg/kg). (*b*) Salicylamide: b = 145.2±20; dose for 50% protection = 126.6 mg/kg (95% confidence limits, 86.2–185.8 mg/kg). (*c*) Acetylsalicylic acid: b = 86.4±4.9; dose for 50% protection = 140.0 mg/kg (95% confidence limits, 73.5–266.4 mg/kg). The broken lines represent the standard errors of control values.

DISCUSSION

It has been shown that the incidence of stretching in mice injected intraperitoneally with phenylbenzoquinone may be treated as a graded response, reduction of which by both narcotic and non-narcotic analgesics is a suitable basis for assay of potency.

The slope of the dose/response relationship for acetylsalicylic acid was much less than that for the narcotic analgesics. This may be a consequence of the contribution from an anti-inflammatory component of the action of acetylsalicylic acid in this situation, as shown by Whittle (1964) for stretching due to acetic acid.

Although the performance of the assay at higher temperature did not alter the slope or position of the dose/response relationship of either narcotic or non-narcotic analgesics, working at 34° C did reduce the number of animals required for a given precision. There are two contributions to this; the incidence of response in all mice injected with the agent, and the smaller variance of response. The principal advantage of the use of mice after dieting and fasting has been the steeper slope of the dose/response relationship obtained with non-narcotic analgesics, permitting more reliable estimates of their relative potencies. This steeper relationship is, however, accompanied by reduced effectiveness of lower doses, with a consequent increase in the doses required for 50% reduction of response.

SUMMARY

1. The conditions for precision in the assay of analgesics, by reduction of the response to intraperitoneal injection of phenylbenzoquinone in mice, were investigated.

2. The assay was based on a graded response, stretching movements being counted in groups of five mice over a 5-min period when the rate was maximal.

3. Performing the assay in an external temperature of 34° C reduced the number of mice necessary.

4. Dieting and fasting the mice before use steepened the dose/response relationship and increased the precision of determining the effectiveness of non-narcotic analgesics.

5. The dose/response relationship and precision of determination for narcotic analgesics were not affected by this procedure.

REFERENCES

CARROLL, M. N. & LIM, R. K. S. (1958). Mechanism of phenylquinone writhing. *Fed. Proc.*, **17**, 357.

ECKHARDT, E. T., CHEPLOVITZ, F., LIPO, M. & GOVIER, W. M. (1958). Etiology of chemically induced writhing in mouse and rat. *Proc. Soc. exp. Biol. (N.Y.)*, **98**, 186–188.

HENDERSHOT, L. C. & FORSAITH, J. (1959). Antagonism of the frequency of phenylbenzoquinone-induced writhing in the mouse by weak analgesics and non-analgesics. *J. Pharmacol. exp. Ther.*, **125**, 237–240.

SIEGMUND, E. A., CADMUS, R. A. & GO LU (1957a). Screening of analgesics, including aspirin-type compounds, based upon the antagonism of chemically induced writhing in mice. *J. Pharmacol. exp. Ther.*, **119**, 184.

SIEGMUND, E. A., CADMUS, R. A. & GO LU (1957b). A method for evaluating both non-narcotic and narcotic analgesics. *Proc. Soc. exp. Biol. (N.Y.)*, **95**, 729–731.

VANDER WENDE, C. & MARGOLIN, S. (1956). Analgesic tests based upon experimentally induced acute abdominal pain. *Fed. Proc.*, **15**, 494.

WHITTLE, B. A. (1964). The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics. *Brit. J. Pharmacol.*, **22**, 246–253.