A COMPARATIVE STUDY OF THE WRITHING RESPONSE INDUCED BY PHENYLQUINONE, BRADYKININ AND ACONITINE FOR THE ASSESSMENT OF ANALGESIC AGENTS*

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Accepted February 26, 1973

Abstract—Phenylquinone, bradykinin and aconitine induced writhing syndromes have been compared as to screening suitability for analgesic agents. It was observed that aconitine induced writhing appears more quickly, is more frequent and lasts for a longer period. Drug antagonism studies show that both phenylquinone and bradykinin induced writhing responses and are antagonised by antipyretics as well as narcotic analgesics, while aconitine induced writhing syndrome, is more selectively antagonised by the antipyretic analgesic agents.

A writhing syndrome characterized by abdominal torsion, drawing up of the hind legs to the body, marked contraction of the abdominal area and arching of the back so that the caudal ventral surface of the mouse touched the glass floor, can be induced by an i.p. injection of agents having a diverse chemical nature. Certain iodinated compounds (1), phenylquinone (2, 3), bradykinin (4) adrenaline (5) and aconitine (6) have been reported to elicit the characteristic response. The phenylquinone induced writhing response has been advocated as a test for analgesic activity (2). It has been claimed that the writhing response exhibited by the iodinated compounds can be more selectively antagonized by narcotic analgesics (1) and similarly the bradykinin induced response can be more selectively antagonized by the aspirin type of analgesic agents (4).

The present investigation was carried out to compare the writhing syndromes induced by aconitine, phenylquinone and bradykinin and to determine suitability of these methods for assessment of analgesic activity of narcotic as well as non-narcotic agents. Whittle (7) showed that acetic acid writhing is not a specific test. It does not distinguish between narcotic and non-narcotic drugs and may give a positive result with compounds which are not regarded as clinically useful analgesics.

MATERIALS AND METHODS

The present investigation was carried out on seven hundred and forty adult albino mice weighing between 20 to 25 g. Each mouse was placed separately on a glass surface and observed for 90 min. Graded doses of aconitine (0.5, 1 and 2 μg per animal), phenyl-
quinone (25, 50 and 100 μg per animal) and bradykinin (2, 4 and 6 μg per animal) were injected i.p. and the animals were observed for the onset, frequency and duration of the characteristic writhing response. The results were statistically analysed and the 100% effective dose (ED<sub>100</sub>) of aconitine, phenylquinone and bradykinin for inducing the writhing response was determined.

In another series, the protective effect of graded doses of several known analgesics viz., acetylsalicylic acid, sodium salicylate, phenylbutazone, amidopyrine, morphine, phenacetin, codeine and mephenesin, a central muscle relaxant, were studied against the writhing syndrome induced by aconitine, phenylquinone or bradykinin. Groups of 10 mice per dose of each drug were used. Specific doses of the protective agents were given orally to the animals 30 min prior to the dose (ED<sub>100</sub>) of the above mentioned writhmogenic agents. The animals were observed for the development of writhing response upto one hr. The appearance of even one writhing movement per mouse was considered positive. The results were statistically analysed and the PD<sub>50</sub> values of each drug was calculated against different writhmogenic agents.

Aconitine was dissolved by adding one drop of concentrated hydrochloric acid and diluted with saline to give a desired concentration. Phenylquinone was dissolved in 5% ethyl alcohol and distilled water. The synthetic bradykinin (Sandoz, Basel) was dissolved in distilled water. The volume for i.p. injection never exceeded 0.1 ml.

RESULTS

Control studies

Following the intraperitoneal injections of phenylquinone, bradykinin and aconitine each mouse was observed to display the characteristic writhing syndrome. Characteristics of the syndrome were: torsion of abdomen, drawing up of the hind legs to the body, marked contraction of the abdominal musculature and arching of the back so that the caudal ventral surface of the mouse touched the glass floor. The features of the writhing syndrome with phenylquinone, bradykinin and aconitine were the same and were dose-dependent. The ED<sub>100</sub> for inducing the writhing syndrome with phenylquinone, bradykinin and aconitine was 100 μg, 4 μg and 2 μg respectively (Fig. 1). The writhing response with phenylquinone (ED<sub>100</sub>=100 μg i.p.) appeared 10 min after administration and persisted for a period of 90 min. The total number of writhes in each mouse was approx. 20 to 30. The peak effect was observed between 40 and 50 min after the injection.

With ED<sub>100</sub> of bradykinin (4 μg i.p.) the writhing response developed 6 min after injection of the agent and persisted for 40 min. The total number of writhes in each mouse was approx. 6 to 12 during the observation period. The peak effect was observed between 6-10 min. Aconitine (ED<sub>100</sub>=2 μg i.p.) induced writhing response within 2 min of administration and the response lasted for 60 min. The total number of writhes observed were 35 to 45. The maximum number of writhes with aconitine was seen during the first half hr. Results are shown in Fig. 2.
Drug studies

The protective effect of graded doses of the known analgesic agents viz. acetylsalicylic acid, sodium salicylate, phenylbutazone, amidopyrine, phenacetine, morphine, codeine and mephenesin was studied against the writhing response induced by aconitine, bradykinin and phenylquinone. Results are shown in the Table 1.

It can be noticed from the results that acetylsalicylic acid a antipyretic analgesic has approx. the same PD₅₀ value against all three writhmogenic agents. Other antipyretic analgesics viz. sodium salicylate, phenylbutazone, amidopyrine and phenacetine showed different PD₅₀ values against the three writhmogenic agents. On the other hand, the PD₅₀ value of morphine against aconitine writhing syndrome was approx. three times that required against phenylquinone and bradykinin writhings. The PD₅₀ value of codeine against aconitine writhing was approx. seventeen times more than bradykinin and five times more than phenylquinone writhing. Mephenesin is more effective in blocking phenylquinone and bradykinin writhing whereas it is ineffective against aconitine writhing.

FIG. 1. Regression lines showing log doses (µg per mouse i.p.) and percent writhing response induced by aconitine, bradykinin and phenylquinone.

FIG. 2. Bar diagrams showing number of writhes ± S.E. onset and duration of the response at 100% effective doses of phenylquinone, bradykinin and aconitine in albino mice.
DISCUSSION

Experimental methods for testing the analgesic activity are based on the production of pain by mechanical, thermal, electrical and chemical stimuli. Most of these methods are unsuitable for screening of acetylsalicylic acid (aspirin) type of analgesic agents. Writh-
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ing syndrome induced by agents of diverse nature has been claimed suitable for screening of analgesic drugs. The present investigation was carried out with an idea of comparing phenylquinone, bradykinin and aconitine induced writhing syndrome(s) and to determine suitability as a method for screening of newer analgesic agents. Scherf (8) used aconitine for the production of cardiac arrhythmias. Kohli and Bryant (9) established the membrane depolarising action of aconitine. Bhargava et al. (10) used this agent intracerebroventricularly (i.c.v.) for the production of cardiac arrhythmias. Considering its strong depolarising action at various membrane levels it was thought worthwhile to use this agent intraperitoneally for producing writhing response.

It is evident from (Fig. 2) that aconitine induced writhing is quicker to appear, showed greater frequency as compared with those of bradykinin and phenylquinone writhing syndrome. The duration of aconitine writhing (60 min) was long enough to assess the analgesic activity of new compounds. Parkes and Pickens (11) have also studied the time course of phenylquinone induced stretching responses. Duration of action of these experiments (7) was 70 min, however, they reported a relatively greater number of responses during the course of observation. The discrepancy may be due to the fact that they counted the stretching of hind limbs and not the characteristic writhing response.

The results of this study show that phenylquinone writhing syndrome can be antagonized by aspirin as well as morphine type of analgesics. On the basis of similar results Siegmund et al. (2) suggested that phenylquinone writhing syndrome be a good model for testing analgesic activity of both types of agents. Bradykinin induced writhing has been claimed relatively more specific for screening antipyretic analgesics and anti-inflammatory agents (4), however, in the present study morphine was found to be effective in antagonizing the bradykinin induced writhing also in the doses (PD₅₀ = 23.4 ± 0.26 mg/kg) which antagonized the phenylquinone (PD₅₀ = 24.6 ± 0.24 mg/kg) writhing. Similarly codeine antagonized the bradykinin and phenylquinone writhings in albino mice in doses (PD₅₀ values 3.2 ± 0.12, 10.4 ± 0.30 mg/kg respectively).

Moreover, Emele and Shanaman (4) and Parkes and Pickens (11) also observed the antagonism of bradykinin induced writhing by codeine. The shelf-life of bradykinin is short; and this is a positive drawback regarding usage. Aconitine induced writhing on the other hand, is antagonized by antipyretic analgesic and by very high doses of morphine and codeine (PD₅₀ = 75.9 ± 0.12; 54.6 ± 0.20 mg/kg respectively). Moreover, in an earlier report we have shown that non analgesic CNS active agents viz. methaqualone, dilantin, imipramine, mephenesin and phenoxylbenzamine did not antagonize aconitine induced writhing (6). Thus, aconitine induced writhing shows greater selectivity for the screening of antipyretic analgesics.

Acknowledgements: The authors are grateful to the Council of Scientific and Industrial Research, New Delhi for financial assistance and to Mr. O.S. Tewari for technical assistance.
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