A SIMPLE NEW QUANTITATIVE METHOD FOR TESTING LOCAL ANAESTHETICS

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Local anaesthetic activity is usually determined either by the method originally described by Regnier (1923) and later modified by other authors (inhibition of the corneal reflex by local application of drug), or by the method of Bülbring and Wajda (1945) (inhibition of the reaction to pin-prick stimulus of the skin by intracutaneous injection of drug).

The first method measures surface anaesthetic activity ; the second method also tests the power of infiltration. The power of the local anaesthetic to block nerve conduction is much less frequently determined. Either the method of Bülbring and Wajda (1945) (inhibition in the decapitated eviscerated frog of reflex contraction of the leg after stimulation by immersion in HCl, after injection near the plexus ischiaticus), Shackell's method (1935) (inhibition in the guinea-pig of the pain reflex provoked by stimulation of the foot after injection near the nervus ischiaticus), or the method of Dietrichs (1931) (inhibition of the reflex contraction to electric stimulation of the ischiaticus gastrocnemius preparation of the frog with local application of the drug to the nerve), may be used.

Bianchi (unpublished) measures local anaesthetic activity by the method reported by Kisch (1948), but using *Rana temporaria* instead of the bullfrog. The frogs lose their righting reflex for a certain period of time after intracranial injection of the drug. Unfortunately, however, this method is not specific, since hypnotics such as phenobarbitone produce the effect as well as local anaesthetics.

Mack and Nelson (1953), and Herr, Nyiri, and Pataky (1953), applied to local anaesthetics the radiant heat method of Hardy, Wolff and Goodell (1940) for the determination of analgesic activity. The time required for the reappearance of the pain reflex in the rat's tail is measured after subcutaneous injection of the compound.

In order to overcome some difficulties encountered during the application of the above methods in screening new compounds with supposed local anaesthetic activity, we thought it desirable to try Haffner's method (1929) (application of pressure on the mouse tail's root), which, as demonstrated by Bianchi and Franceschini (1954), is suitable for the quantitative estimation of analgesic drugs.

METHOD

Fully grown albino mice of either sex may be used. A small artery clip with its blades covered by thin rubber tube is applied to the root of the tail. Those animals which do not show within 30 sec. the pain reflex (the mice turn again and again trying to remove the clip) are eliminated. The remainder receive subcutaneously, about 1 cm. from the root of the tail, 0.1 ml. of a solution of the drug. 15 min. after injection the pain reflex of all the injected animals is tested, applying the stimulus to the zone where the compound was injected.

The proportion of animals which proved to be anaesthetized (animals which within 30 sec. after the application of the clip do not show the usual pain reflex) was noted for each dose.

The following drugs were tested in 2% (w/v) solution : cocaine hydrochloride, procaine hydrochloride, lidocaine (lignocaine) hydrochloride, and cinchocaine hydrochloride.

These solutions were further diluted with saline to obtain the concentration desired.

To all solutions adrenaline hydrochloride (10 μg./ml.) was added.

RESULTS

Injections of saline solutions with adrenaline do not affect the pain reflex.

There is a linear relation between the log of the concentration of local anaesthetic and the probit of the percentage of mice showing anaesthesia (Fig. 1). The slopes of the dose-response lines obtained with different local anaesthetics may be regarded as parallel ($P=0.05$). The ED50, and S, with fiducial limits, have been calculated graphically for each drug by the method of Litchfield and Wilcoxon (1949). The results obtained are shown
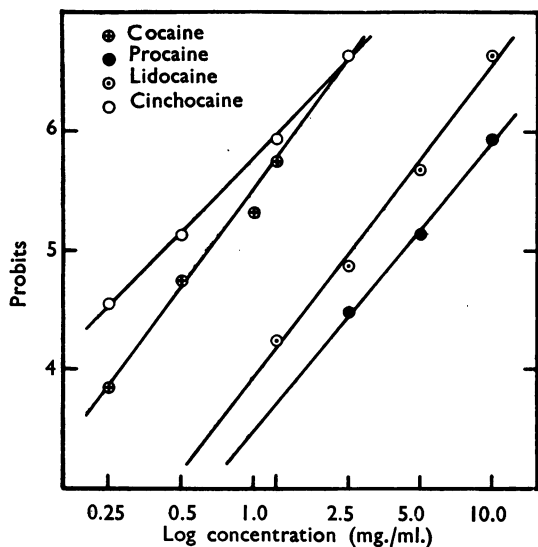
FIG. 1.—Showing the anaesthetic activity of various drugs when tested 15 min. after subcutaneous injection to tails of mice. Abscissa, log concentration in mg./ml. Ordinate, probits.

in Table I. The ED50 of cocaine in this series was 0.66 mg./ml. In another group of 75 mice the ED50 of cocaine was 0.70 mg./ml. (with fiducial limits of 0.58–0.84 mg./ml.), which agrees well with the first result. The activity of commercial preparations of procaine was tested and the results showed no significant difference from those of pure procaine HCl (Table II).

The anaesthetic activity of all these compounds is of short duration and wears off within 90 min.

The activity of cocaine is nearly half that of cinchocaine, approximately four times that of lidocaine, and seven times that of procaine. The activity of lidocaine is about twice that of procaine.

TABLE I

LOCAL ANAESTHETIC ACTIVITY TESTED 15 MIN. AFTER SUBCUTANEOUS INJECTION OF THE DRUGS TO TAILS OF MICE

(The numerals in parentheses are the 19/20 fiducial limits)

Drug	No. of Mice	ED50 (mg./ml.)	S	Activity Ratios (Cocaine = 1)
Cocaine HCl ..	160	0.66 (0.53–0.81)	2.31 (1.67–3.18)	1
Procaine HCl ..	120	4.25 (3.40–5.31)	2.56 (1.69–3.86)	0.15 (0.10–0.20)
Lidocaine HCl	160	2.60 (2.00–3.20)	2.40 (1.81–3.16)	0.25 (0.18–0.34)
Cinchocaine HCl	140	0.42 (0.31–0.55)	3.02 (2.01–4.53)	1.57 (1.20–2.29)

DISCUSSION

The potency ratios of local anaesthetics obtained with this new method agree with those obtained by some other methods (Goodman and Gilman, 1955 ; Krantz and Carr, 1954), but not always with those reported by other authors,—for example, Mongar (1955). It may be that differences in the technique used account for the discrepancies in the activity ratios found. Indeed, the presence of buffer solutions such as phosphate increases the activity of the local anaesthetics. Mongar showed

TABLE II

LOCAL ANAESTHETIC ACTIVITY TESTED 15 MIN. AFTER SUBCUTANEOUS INJECTION OF COMMERCIAL SAMPLES OF PROCAINE TO TAILS OF MICE

(The numerals in parentheses are the 19/20 fiducial limits)

Sample	No. of Mice	ED50 (mg./ml.)	S	Activity Ratios (Procaine = 1)
Procaine HCl ..	120	4.25 (3.40–5.31)	2.56 (1.69–3.86)	1
1	180	5.80 (4.00–8.40)	6.20 (2.60–14.2)	0.73 (0.47–1.12)
2	160	5.90 (4.70–7.40)	2.14 (1.62–2.76)	0.72 (0.53–0.97)
3	140	4.40 (3.57–5.41)	1.87 (1.55–2.24)	0.96 (0.71–1.29)
4	60	5.00 (3.33–7.50)	3.21 (1.14–8.98)	0.85 (0.53–1.35)

that, when the drugs are administered in buffered solutions, cinchocaine is 34 times more active than procaine by the guinea-pig weal method. Elio (1948), with the same technique but using unbuffered solutions, found cinchocaine to be only ten times more active than procaine.

The technique described in this paper offers the advantages of easy and rapid performance, and of results which are both quantitatively and readily compared. The performance of the test does not require any particular ability ; even the quantity of solution to be injected does not have to be kept absolutely constant. Indeed, there is frequently leakage from the site of injection owing to the high local pressure. During the great number of tests performed, only once have we encountered a dose response line with a slope less than that usually found: the reason for this was not apparent.

SUMMARY

1. A simple new method for testing local anaesthetic properties of drugs on mice is described.

2. The activities of cocaine, procaine, lidocaine, and cinchocaine were compared ; the results agree with those quoted by other authors.

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