Journal of Medicinal and Pharmaceutical Chemistry

VOL. 3, No. 3 (1961)

# Experimental Evaluation of the Activities of Some Local Anaesthetics with Particular Reference to a New Derivative: Chloroparidocaine*

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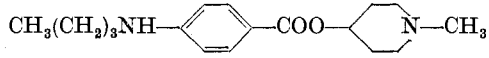
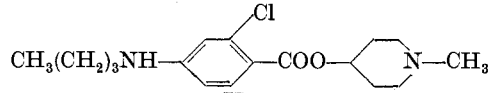
A long-acting local anaesthetic, paridocaine[1] (I) (*N*-methyl 4-piperidyl *p*-*n*-butylaminobenzoate), has recently been introduced into clinical practice.



(I)

As nuclear substitution of chlorine into procaine more than doubles the activity,[2] reduces the latent period, and confers some bacteriostatic activity,[3] the corresponding chloroparidocaine (II) was prepared:[4]



(II)

This compound was isolated as colourless plates, m.p. 61°, b.p. 240–243°/0·5 mm; it was soluble in the common organic solvents and insoluble in water. The methanesulphonate, m.p. 146° (colourless blades), which was soluble in water, was used in the pharmacological studies to be described. The hydrochlorides of procaine and its chloro derivative were also compared pharmacologically.

Three types of tests were used to evaluate pharmacological activity: the rabbit's cornea test,[5] the saturation P.D. of the frog's isolated sciatic nerve, and counting the number of responses of

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frog's tongue receptors immersed in a solution of calcium chloride. The last test is based on the observation by Casella and Rapuzzi[6] that the pharingo-glottal nerve of the frog's tongue produces intense action potentials when immersed in solutions of calcium chloride containing only $0 \cdot 013 \times 10^{-3}$ moles of $CaCl_2$.

## Experimental

(*a*) *Toxicity in guinea pigs.* Aqueous solutions of the compounds were introduced by intravenous drip. Paridocaine has also been tested in the form of methane sulphonate, which is obtained by dissolving the pure base in the calculated quantity of methanesulphonic acid in water. The carotid pressure and respiration were recorded simultaneously. Fall of pressure to zero or cessation of breathing were taken as an indication of the death of the animal.

(*b*) *Surface anaesthesia and pruritic activity of the anaesthetics on the rabbit's cornea.* The method described by Koelzer[5] was used. The duration of anaesthesia after dropping $0 \cdot 25$ ml of a 2 per cent solution of the test substance was measured. Anaesthesia was indicated by the disappearance of corneal reflex to stimulation of the ocular surface by a glass rod.

We assessed the pruritic activities of the anaesthetics by examining the conjunctiva 3 and 24 hours after treatment. The observed alterations are classified in four degrees: hyperaemia, cellular exudation, blisters and vesicles, and ulcers with irreversible scar damage.

(*c*) *Action potentials of the frog's sciatic nerve.* The nerve was isolated along its entire length and placed on a series of silver electrodes in a humid, oxygenated chamber. The cranial end of the nerve was stimulated by rectangular current impulses of $0 \cdot 2$ msec duration and at a frequency of 20 per second. The action potentials were measured by another pair of electrodes placed about 2 cm from the site of stimulation and connected to a Tektronix oscillograph. Throughout the experiment, the intensity of the stimulus was greater than that required for maximal response.

The intensities of the action potentials were measured both immediately after preparation of the nerve, and after the nerve had

been immersed for 10 min in oxygenated Ringer's solution containing the compound under examination. The measurements were then repeated every 10 min up to a maximum period of 100 min. The nerve was kept in oxygenated Ringer's solution at room temperature between measurements. The intensity of the action potential was taken as indicating the number of fibres remaining active after the action of the anaesthetic. The intensities of the action potentials have been expressed as percentages of the initial values.

(*d*) *Frog's tongue receptors.* The experiments were carried out on *Rana esculenta* during winter hibernation. The animals, beheaded and with the spinal cord removed, were placed on the type of block described by Casella and Rapuzzi;[6] the tongue was placed in a glass test tube into which the solutions under examination were transferred. The pharingo-glottal nerve, isolated over a length of about 2 cm, was placed on two platinum electrodes connected to a differential amplifier. The action potentials were shown on the screen of a cathode ray oscillograph and could be photographed. They were also counted electronically and made audible through a loudspeaker.

After dissection, the animal's tongue was immersed in a solution containing 50 μg of $CaCl_2$ per ml of distilled water and the impulses were counted during 1 min; the $CaCl_2$ solution was then replaced by the solution of anaesthetic which was allowed to act for 5 min; the tongue was then immersed in the $CaCl_2$ solution and the impulses again counted, after which counting was repeated at 10-min. intervals. During these intervals, the tongue was immersed in isotonic NaCl solution which inactivates the receptors.

## Results

The toxicity of each compound was tested on 6 guinea-pigs; the results are shown in Table I.

Table I. Comparison of the toxicity of the various anaesthetics studied (average value ± probable error)

| Procaine | Chloroprocaine | Paridocaine | Chloroparidocaine |
|---|---|---|---|
| 72·8 ± 17·7 mg/kg | 64·8 ± 2·6 mg/kg | 5·6 ± 1·7 mg/kg | 38·8 ± 6 mg/kg |

Duration of anaesthesia on the rabbit's cornea was determined in four groups of six animals each; the degree of local irritation was evaluated in the same animals. The results are shown in Table II.

Table II. Duration of surface anaesthesia and degree of local irritation on rabbit's cornea (average value ± probable error)

| | Procaine | Chloroprocaine | Paridocaine | Chloroparidocaine |
|---|---|---|---|---|
| Duration of anaesthesia, sec | 29 ± 5 | 35 ± 7 | 57 ± 8 | 42 ± 7 |
| Degree of irritation (Koelzer's scale) | | | | |
| After 3 h | 0 | 0 | II | II |
| After 24 h | 0 | 0 | II–III | III |

The effects of the anaesthetics on the excitability and on the conduction of excitation in the nervous trunk were studied on the sciatic nerves of 140 frogs. Each compound was tested at least 30 times and each dilution at least 10 times. Increasing dilutions were chosen to establish the concentration of each substance required to reduce the action potential to about 50 per cent of its original value. The intensity of action potential, measured immediately after dissecting the nerve, was between 10 and 25 mV, and only decreased by about 6 per cent after 2 h, in the control tests.

Table III shows the results of these tests.

In studying surface anaesthesia on the frog's tongue, the concentrations of the anaesthetics were chosen to give comparable effects. The results of the 31 tests carried out are shown in Table IV.

The number of impulses (in the first determination) varied from 5,000 to 20,000 per minute. The fall in activity in control tests was taken into account in evaluating the activities of the anaesthetics.

In animals treated with anaesthetic, the activities of the receptors markedly diminish or disappear, and gradually regain their normal value after a period of time that depends upon the compound used and its concentration.

Table III. Completeness of action potentials of frog's sciatic nerve before and after immersion of the nerve in various concentrations of the solutions of the four anaesthetics under examination (averages of the 10 measurements expressed in percentages of the initial values)

| Substance | Concentration | Initial reading, 0 sec | After action of anaesthetic for 10 sec | After immersion in Ringer's for 10 sec | 20 sec | 30 sec | 40 sec | 60 sec | 80 sec | 100 sec | 120 sec |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Controls | | 100 | | 98 | 98 | 98 | 97 | 97 | 96 | 97 | 94 |
| Procaine | 1/1000 | 100 | 1·4 | 36 | 61 | 79 | 87 | 99 | | | |
| | 1/5000 | 100 | 53 | 87 | 95 | 100 | | | | | |
| | 1/10,000 | 100 | 64 | 86 | 92 | 94 | | | | | |
| Chloroprocaine | 1/5000 | 100 | 15 | 73 | 83 | 90 | 93 | 96 | | | |
| | 1/10,000 | 100 | 42 | 91 | 98 | 101 | | | | | |
| | 1/20,000 | 100 | 71 | 92 | 99 | 99 | | | | | |
| Chloroparidocaine | 1/10,000 | 100 | 36 | 40 | 50 | 58 | 59 | 67 | 70 | 81 | |
| | 1/20,000 | 100 | 43 | 60 | 63 | 69 | 73 | 77 | 83 | 89 | |
| | 1/40,000 | 100 | 59 | 73 | 78 | 83 | 86 | 89 | | | |
| | 1/80,000 | 100 | 68 | 82 | 86 | 90 | | | | | |
| Paridocaine | 1/40,000 | 100 | 34 | 34 | 36 | 38 | 38 | 38 | 38 | 39 | 40 |
| | 1/80,000 | 100 | 37 | 42 | 48 | 54 | 57 | 63 | 67 | 68 | |
| | 1/160,000 | 100 | 47 | 61 | 63 | 68 | 79 | 87 | 89 | 90 | |

Table IV. Action of the anaesthetics on frog's lingual receptors—number of impulses which run along the glotto-pharingal nerve in 1 sec. The averages of the observations made after the action of the anaesthetic are expressed as a percentage of the initial values

| Substance studied | Initial reading, 0 sec | After action of anaesthetic for 5 sec | After immersion in isotonic sol. of NaCl for 10 sec | 20 sec | 30 sec | No. of experiments |
|---|---|---|---|---|---|---|
| Controls | 100 | | 84 | 77 | 69 | 4 |
| Procaine | | | | | | |
| 1/20,000 | 100 | 50 | 82 | | | 5 |
| Chloroprocaine | | | | | | |
| 1/10,000 | 100 | 40 | 46 | 65 | 84 | 5 |
| Paridocaine | | | | | | |
| 1/80,000 | 100 | 23 | 36 | 57 | 63 | 5 |
| Chloroparidocaine | | | | | | |
| 1/20,000 | 100 | 7 | 28 | 47 | 68 | 4 |
| 1/40,000 | 100 | 27 | 28 | 38 | 70 | 3 |
| 1/80,000 | 100 | 43 | 34 | 72 | | 5 |

## Discussion of Results

The surface anaesthesia and blocking effect on nerve trunks caused by paridocaine and chloroparidocaine are notably greater and more prolonged than those of the *p*-aminobenzoates. The frog's tongue test shows particularly clearly that:

(*a*) The concentrations of procaine and chloroprocaine required to lower the activities of the receptors by about 60 per cent are from four to eight times higher than that of chloroparidocaine.

(*b*) Surface anaesthesia caused by chloroprocaine is slightly less intense but more prolonged than that caused by procaine.

(*c*) Paridocaine is about two times more active but six times more toxic than chloroparidocaine.

These observations have been substantially confirmed by the rabbit's cornea test, which indicates that the duration of surface anaesthesia due to chloroparidocaine is slightly shorter, while that due to procaine and chloroprocaine is much shorter than that caused by paridocaine. Procaine and chloroprocaine are less irritant than paridocaine and chloroparidocaine.

The frog's sciatic nerve test also confirms, on the whole, the results of the other tests. In this case, the blocking effect of paridocaine is from four to eight times greater than that of its chloro-derivative. On the other hand, 25 mg/l. of paridocaine seem to cause partial irreversible blockage, which is unchanged after 2 h, possibly due to alteration of the nerve fibres. 100 mg/l. of chloroparidocaine promote similar blockage which is, however, reversed in 2 h.

*Summary.* Determination of toxicity, the rabbit's cornea test (surface anaesthesia and pruritic activity), the frog's sciatic nerve test and counting the number of responses of the frog's tongue receptors using procaine, chloroprocaine, paridocaine and chloroparidocaine, have led to the following conclusions:

(*a*) Surface anaesthesia and the effects on nerves caused by paridocaine and chloroparidocaine are more intense and last longer than those caused by procaine and chloroprocaine. However, these compounds have pruritic activity not observed with the *para*-aminobenzoates that we studied.

(*b*) Paridocaine is twice as potent a surface anaesthetic as chloroparidocaine, but six times more toxic. The therapeutic index of paridocaine is therefore less favourable.

(*c*) Chloroparidocaine is appreciably less active than paridocaine in inhibiting activity of the frog's sciatic nerve. However, paridocaine promotes in some cases irreversible blockages, which might indicate alterations of the nerve.

## References

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