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THE LOCAL ANESTHETIC ACTIVITY OF PROADIFEN HCl (2-DIETHYLAMINOETHYL-α,α-DIPHENYLVALERATE, HCl)

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The direct blocking effect of proadifen on nerve conduction and surface receptors was compared with that of lidocaine and procaine. Proadifen was active as a local anesthetic at significantly lower concentrations than the other two drugs in the desheathed sciatic nerve preparation as well as in the rabbit cornea. Its potency in the isolated nerve is 7.85 times that of procaine.

Proadifen local anesthetic activity Procaine Lidocaine Desheathed sciatic nerve

1. INTRODUCTION

The compound proadifen, HCl (2-diethylaminoethyl-α,α-diphenylvalerate HCl or SKF 525-A) is an enzymatic inhibitor and much of the work reported on this substance is concerned with its ability to interfere with a wide variety of hepatic microsomal drug biotransformations (Brodie et al., 1958). The local anesthetic action of proadifen was first observed by Burt et al. (1967) whilst studying the activation of diazinon on cockroach ganglia, but was not fully described.

We studied proadifen as a conduction blocking agent on nerve axons and on surface receptors and compared its activity with that of lidocaine and procaine.

2. METHODS

2.1. *Conduction blocking effect*

Blocking conduction activity was assessed in a desheathed nerve preparation according to a previously described method (Guerrero, 1964; Staff of the Department of Pharmacology, University of Edinburgh, 1968). Sciatic nerves of the giant toad *Calyptocephalella gayi* were used. The connective-tissue sheath was carefully stripped away under a microscope and the preparation was kept in a moist air chamber at room temperature.

The compound action potentials evoked by supramaximal stimulation were rendered monophasic and recorded by a conventional electrophysiological method. The amplitude of the compound action potentials were measured directly on the screen of a Tektronix oscilloscope before and after adding drugs.

Each preparation was used for 2 to 4 determinations by washing the drug out and waiting for the complete recovery of the action potential amplitude. Each drug concentration was studied on 4 to 12 nerves. The results were expressed as the percentage decrease of the action potential amplitude after 20 min of exposure to the drug and plotted in a probit-logarithmic paper graph. The $ED_{50} \pm$ its standard error for each drug was estimated by the method of Miller and Tainter (1944). The threshold effective concentration (ED_5) for each compound was also estimated from the dose-response curves.

2.2. *Surface anesthetic effect*

The local anesthetic activity on superficial receptors was determined on the rabbit cornea as described by Lechat (1955).

The test solutions were instilled into the conjunctival sac and left there for 2 min. Stimuli were applied to the cornea by pressure from a nylon hair stimulator at a frequency of about 2 per second until the oculo-palpebral reflex was evoked (maximum 100 per stimulation period). Each period of stimulation consisted of 100 stimuli, or less if the oculo-palpebral reflex was evoked. An interval of not less than 5 min separated two stimulation periods.

The intensity of the anesthetic action was expressed as the total number of stimuli that could be applied to the cornea from the administration of the anesthetic solution until the reappearance of the oculo-palpebral reflex. Obviously, the method also determined the duration of the effect.

Eight to twelve experiments were performed at each drug concentration.

Proadifen HCl (2-diethylaminoethyl-α,α-diphenylvalerate hydrochloride, SKF 525-A), lidocaine HCl and procaine HCl were used in saline solutions and their pH values adjusted with 1 N NaOH or HCl to 6.70, 6.50 and 6.89 respectively.

3. RESULTS

3.1. *Proadifen blocking activity on axonal conduction*

The dose-effect relations of proadifen, lidocaine and procaine on the amplitude of the monophasic compound action potential of the toad desheathed sciatic nerve are shown in fig. 1. This figure shows, in a logarithmic-probit scale, that the effects of the three compounds are dose-related, proadifen being by far the most active of the three drugs studied.

The threshold effective concentrations (ED_5) i.e. the drug concentration that inhibits by 5% (Probit 3.35) the compound action potential amplitude after 20 min of exposure was about 1.2×10^{-5} M for proadifen, 5.7×10^{-5} M for lidocaine and 6.7×10^{-4} for procaine. Proadifen 2×10^{-3} M (more than 100 times the threshold dose) completely blocked conduction and even after washing, the initial action potential amplitude did not return in contrast to recovery from concentrations of lidocaine or procaine.

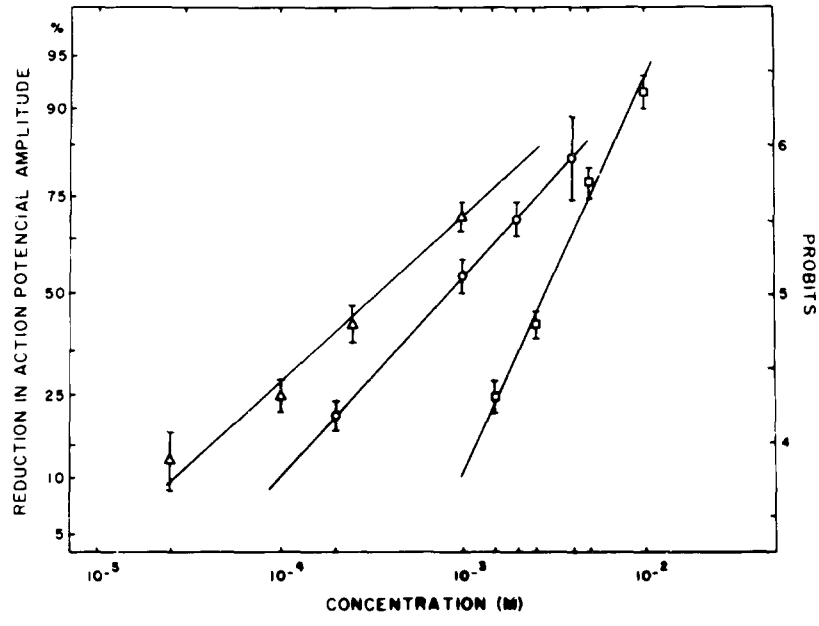

Fig. 1. Effects of axonal conduction of proadifen HCl (△), lidocaine HCl (○) and procaine HCl (□). Percentage amplitude reduction of the compound action potential after 20 min exposure. At right, probit scale. Each point represents the mean ± S.E. of probits of 8 to 24 determinations.

The ED_{50} ± its standard error for the three drugs as well as their equiponent molar ratios at the ED_{50} level are summarized in table 1. According to these figures, proadifen appears to be 7 to 8 times as active as procaine and about 3 times as potent as lidocaine.

The time course of action and of recovery after ED_{50} was not significantly different for the three drugs.

Table 1
Activity of proadifen HCl, lidocaine HCl and procaine HCl on the desheathed sciatic nerve of the toad *Caliptocephallela gayi*.

Drug	*N*	ED_{50} ± S.E. (mM)	Equipotent molar ratio *
Proadifen	4 (32)	0.35 ± 0.25	7.85
Lidocaine	4 (35)	0.85 ± 0.14	3.23
Procaine	4 (57)	2.75 ± 0.53	1.00

N = number of doses. The number of determinations are indicated in brackets.
* Relative to procaine.

3.2. *Local anesthetic effect of proadifen on surface receptors*

Results obtained on the rabbit cornea are shown in fig. 2. Proadifen was studied in concentrations ranging from 0.5 mg/ml to 2 mg/ml. The intensity of the anesthetic action was expressed as the sum of the stimuli applied on the corneal surface until the reappearance of the blinking reflex. The duration of the anesthetic effect was also plotted against the logarithm of the concentrations tested. The three compounds showed a linear concentration-effect relationship on both parameters. Proadifen was more active than lidocaine and procaine. Procaine is only poorly absorbed from the surface membrane of the cornea.

There were no significant differences between the three substances concerning the onset, time course of action and of recovery. The corneal reflex recovered without any detectable damage of the mucous surface after proadifen 2 mg/ml.

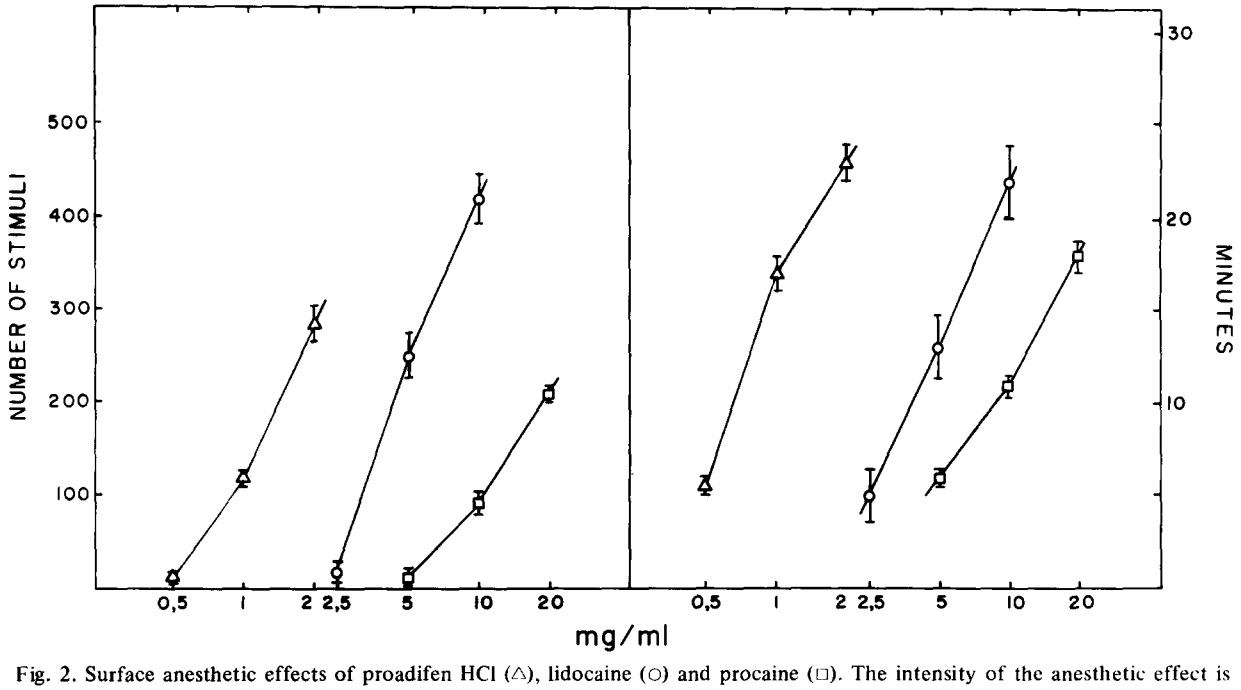

Fig. 2. Surface anesthetic effects of proadifen HCl (△), lidocaine (○) and procaine (□). The intensity of the anesthetic effect is expressed as the total number of stimuli that fail to induce the oculo-palpebral reflex with each concentration. At right, the duration in minutes for the respective effects. Each point represents the mean ± its standard error of 8 to 12 experiments.

4. DISCUSSION

Most papers concerning proadifen show that this substance is devoid of direct effects and that its actions are mainly at the microsomal enzymatic level. Our experiments and those reported by Burt et al. (1967) indicate that this compound exerts an important direct effect at fairly low concentrations on the peripheral nervous system as a conduction blocking agent.

A direct action of proadifen at the neuromuscular junction has also been suggested (Harris and Milton, 1961; Suarez-Kurtz and Paulo, 1968), since this drug inhibits muscle contraction after nerve stimulation.

The data presented in this paper concerning the local anesthetic action of this compound agree with the incidental observation made by Burt et al. (1967) in the course of a study on activation of diazinon by cockroach ganglia. These authors obtained complete block of conduction in giant fibres with 2×10^{-4} M after a long exposure to the drug (90 – 208 min), while the minimum active concentration (ED_5) found in our experiments was about 1×10^{-5} M and the ED_{50} 3.5×10^{-5} M.

These results are not surprising in view of the close structural relationship between proadifen and common local anesthetics, as may be seen in the following formulae:

$NH_2.Ph.CO.O.CH_2.CH_2.N.Et_2$ (Procaine)
$Ph_2.C\,(Pr).CO.O.CH_2.CH_2.N.Et_2$ (Proadifen)

Also, it has been shown that proadifen acts as a biological membrane stabilizer in low concentrations, while in higher concentrations its effect is deleterious (Lee et al., 1968). In fact, these authors reported that proadifen at 10^{-9} to 10^{-4} M concentrations protects the red cell against hypotonic NaCl solutions through a physico-chemical action on the membrane rather than by enzymatic inhibition of the active transmembrane transport. This stabilizing effect on the membrane is shared by local anesthetics, phenothiazine tranquilizers, some barbiturates and steroids.

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