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THE ANAESTHETIZATION OF THE RABBIT'S CORNEA BY NON-SURFACE ANAESTHETICS*

BY

MARGARET E. GREIG, WILLIAM C. HOLLAND,† AND PHILIP E. LINDVIG†

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville 4, Tennessee

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It is well known that there is a great difference among local anaesthetics, some causing a high degree of anaesthesia when applied to mucous surfaces while others have little if any effect although they may be very active infiltration anaesthetics. Procaine (β-diethylaminoethyl-*p*-aminobenzoate hydrochloride) and butyn (γ-di-*n*-butylaminopropyl *p*-aminobenzoate sulphate) may be taken as typical examples of such substances, butyn being an excellent surface anaesthetic, while procaine has little if any effect.

It was thought that these differences might be due to differences in the permeability of mucous surfaces to these substances, which could be correlated with their effects on cholinesterase activity. It has already been shown in this laboratory that inhibitors of cholinesterase activity increased the permeability of erythrocytes and the haemoencephalic barrier (Greig and Holland, 1949a, b, c; Holland and Greig, 1950). If anaesthetics having surface activity penetrate the mucous membrane by virtue of their inhibitory action on cholinesterase, then substances showing active infiltration anaesthesia but no surface anaesthesia might be made to cause surface anaesthesia by blocking cholinesterase activity with physostigmine. This was found to be the case, as shown in the following experiments.

METHODS

The rabbit's cornea *in vivo* was used as a test material for surface anaesthesia. Absence of the corneal reflex when the cornea was touched with a small wire was taken as the criterion of anaesthesia. The drugs‡ were applied to the cornea by one worker and results were recorded by two other observers who were not informed of the drug or combination of drugs applied. When there was a difference of opinion on the effect produced, the results were recorded as being negative. This occasionally occurred within one or two minutes after application of the solution of the drug, but after two or three minutes the opinions were usually the same. The concentra-

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† Research Fellow, U.S. Public Health Service.

‡ Orthoxine (N-methyl-α-methyl-β-*o*-methoxyphenyl ethylamine hydrochloride), mesidocaine (*N-sym*-trimethylphenyl-diethylaminoacetamide hydrochloride), and pyrrolocaine (β-N-thiazole-ethyl ester of *p*-propoxybenzoic acid) were kindly supplied by the Upjohn Company.

tion of local anaesthetic was 1 or 2 per cent (w/v) in water as indicated in Table I. The concentration of physostigmine was 1 mg./c.c.

For experiments *in vitro* on the effects of local anaesthetics on cholinesterase activity, dog erythrocytes were used as a source of enzyme. The final concentration of acetylcholine was 0.01 M ; the concentrations of local anaesthetics and the medium used are indicated in Table II.

Cholinesterase activity was determined by the Warburg technique. Inhibitions of 10 per cent or less were not considered significant.

RESULTS

Table I shows the results of experiments in which the local anaesthetics procaine, orthoxine, and mesidicaine were tested alone and in combination with physostigmine for their effect on the corneal reflex of the rabbit. These anaesthetics are usually

TABLE I

EFFECT OF LOCAL ANAESTHETICS WITH AND WITHOUT PHYSOSTIGMINE ON THE CORNEAL REFLEX OF THE RABBIT

Drug	No. of eyes tested	Number of eyes showing	
		Positive reflex	Negative reflex
		(%)	(%)
Procaine (2%)	15	12 (80)	3 (20)
Orthoxine (2%)	9	8 (89)	1 (11)
Mesidicaine (1%)	6	4 (66)	2 (33)
Physostigmine (0.1%)	15	15 (100)	0 (0)
Procaine + physostigmine	18	2 (11)	16 (89)
Orthoxine + physostigmine	24	1 (4)	23 (96)
Mesidicaine + physostigmine	6	0 (0)	6 (100)

considered to have little surface activity. However, under our experimental conditions some degree of anaesthesia was occasionally found, the corneal reflex being abolished in 20, 11, and 33 per cent of the tests with procaine, orthoxine, and mesidicaine respectively. Physostigmine alone had no effect on the corneal reflex. When the local anaesthetics were applied to the cornea with or after physostigmine the corneal reflex was abolished in 89 per cent of the tests with procaine, 96 per cent with orthoxine, and 100 per cent with mesidicaine.

It was observed that the onset of anaesthesia was almost immediate if the local anaesthetic were applied to the eye in which constriction of the pupil by physostigmine was apparent. When the physostigmine and local anaesthetic were applied together anaesthesia was not observed until two or three minutes after application of the drugs or until there was some pupillary constriction.

Table II shows the results of experiments in which the local anaesthetics procaine, orthoxine, mesidicaine, nupercaine (diethyl-aminoethylamide of 2-*n*-butoxy-cinchonic acid), pyrrolocaine, and butyn were tested for their effect on the activity of the cholinesterase of dog erythrocytes. The first three anaesthetics, as mentioned previously, have little or no surface activity ; the last three will produce anaesthesia

TABLE II

EFFECT OF LOCAL ANAESTHETICS ON THE CHOLINESTERASE ACTIVITY OF DOG ERYTHROCYTES

Each Warburg vessel contained 0.3 c.c. packed dog erythrocytes, 0.5 c.c. sodium bicarbonate (3.5 g./100 c.c.), and 0.2 c.c. acetylcholine (0.1M) in a final volume of 2 c.c. The final concentration of the drug was as indicated.

Drug	Number of experiments	Concentration of drug		
		4×10^{-4}M	8×10^{-4}M	16×10^{-4}M
		% effect		
Procaine	6	0	+5*	−2*
Orthoxine	4	0	−5*	−18
Mesidicaine	4	−6*	−4*	−14
Nupercaine	4	−11	−19	−25
Pyrrolocaine	2	−12	−23	−44
Butyn	3	−47	−61	−68

* Values of less than 10% are not considered significant.

when applied to mucous membranes. It may be seen that procaine, orthoxine, and mesidicaine had no significant effect on cholinesterase activity in the two lower concentrations while nupercaine, pyrrolocaine, and butyn produced significant inhibitions in the same concentrations. In the highest concentration, 1.6×10^{-3}M, some inhibition was produced by orthoxine and mesidicaine, but the degree of inhibition was considerably less than for the group which exhibits surface activity.

Discussion

From the results presented it may be seen that certain local anaesthetics which do not normally anaesthetize mucous membranes may be made to do so by the previous or simultaneous application of physostigmine.

Although we have not proved that for the mucous membrane of the eye physostigmine increases the permeability of the epithelial cells, thus allowing the non-surface anaesthetic to penetrate to the nerve endings, we have previously demonstrated a relationship between permeability and the activity of the acetylcholine-cholinesterase system for other membranes. When cholinesterase activity of red cells is inhibited by physostigmine, marked increases in their permeability to both sodium and potassium occurred (Holland and Greig, 1950). Physostigmine also caused an increase in the rate of onset of convulsions in frogs treated with acid fuchsin presumably by increasing the permeability of the haemoencephalic barrier (Greig and Holland, 1949c).

If surface anaesthesia depends on penetration of the epithelial cells by the local anaesthetic and if the ability to penetrate depends on the inhibitory effect on cholinesterase, one would expect that agents which have surface activity would inhibit this enzyme while agents without surface activity would have no effect. This was indeed found to be the case. Procaine, orthoxine, and mesidicaine had little or no inhibitory effect, while nupercaine, butyn, and pyrrolocaine inhibited cholinesterase activity in concentrations of the order of 10^{-3}M. Payot (1946) has reported that

the local anaesthetics cocaine, panthesin, diocain, and holocaine inhibited erythrocyte cholinesterase activity. These anaesthetics all show surface activity. As far as we are aware the presence of cholinesterase has not been demonstrated in the conjunctival epithelium, but its presence has been shown in various other parts of the eye (Bruckner, 1943a, b ; Koelle and Friedenwald, 1950 ; Plattner and Hintner, 1930 ; Unvas and Wolff, 1937).

SUMMARY

1. Procaine, orthoxine, and mesidicaine, which do not ordinarily produce local anaesthesia when applied to the surface of the cornea, may be made to do so by previous or simultaneous application of a physostigmine solution.

2. This effect of physostigmine may be due to a change in permeability of the cornea by inhibition of cholinesterase activity.

3. Procaine, orthoxine, and mesidicaine, which have no surface activity, had little or no inhibitory effect on erythrocyte cholinesterase activity in concentrations of 4×10^{-4} to 1.6×10^{-3}M.

4. Nupercaine, butyn, and pyrrolocaine, which are surface anaesthetics, inhibited cholinesterase activity in concentrations of 4×10^{-4} to 1.6×10^{-3}M.

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