STUDIES ON LOCAL ANÆSTHETIC DRUGS

BY Y. K. SINHA

*From the Department of Pharmacology, Darbhanga Medical College, Bihar, India**

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MANY workers have produced indirect evidence that acetylcholine may be concerned in transmission at sensory nerve endings (Harvey, Lilienthal and Talbot[1], Gray[2]). A group of drugs of widely different chemical nature yet all possessing local anæsthetic activity has been shown by Elio[3] (1948) to antagonise the action of acetylcholine on different tissues. Local anæsthetic drugs also antagonise the actions of nicotine and potassium chloride on different tissues and abolish the peristaltic reflex in the guinea-pig ileum (Dawes[4], Feldberg and Lin). It was decided, therefore, to study in detail the relative antagonism of a series of 6 well-known local anæsthetic drugs on the responses of skeletal and plain muscle to various stimulants including acetylcholine, nicotine and potassium.

METHODS

The isolated rectus abdominis muscle of the frog, suspended in a bath of volume 2 ml., has been used for experiments on skeletal muscle. The muscle was immersed in a solution of tetraethyl pyrophosphate (3·5 μg./ml.) for 30 minutes to inhibit cholinesterase, and then maintained in a concentration of 0·02 μg./ml. (Hobbiger[6], 1950). Pieces of rabbit and guinea-pig ileum were suspended in an isolated organ bath (volume 15 ml.) containing oxygenated Tyrode's solution at 37° C. A larger bath (volume 50 ml.) was necessary for studying the peristaltic reflex (Feldberg and Lin[5]). Isolated tracheal preparations of the cat and guinea-pig and preparations of the human bronchus (obtained from the post-mortem room) were suspended in a 15 ml. bath (Akcasu[7]).

The local anæsthetic drugs were used in the form of hydrochlorides and the amounts indicated in the text and tables refer to their corresponding salts. In all cases, the values quoted in the tables are the geometric mean of at least 4 results based on a 50 per cent. inhibition of the contraction produced by the stimulant drug.

Other drugs used were acetylcholine chloride, benzoylcholine chloride, succinylcholine bis-iodide, *d*-tubocurarine chloride, nicotinic acid tartrate and histamine acid phosphate.

RESULTS

Frog rectus abdominis muscle

Low concentrations of local anæsthetic drugs (5×10^{-5} to $2{\cdot}5 \times 10^{-6}$) inhibit the acetylcholine and succinylcholine responses on the rectus

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muscle and there is good agreement between relative activities based on these actions and their local anæsthetic potencies (Table I). Whereas procaine and lignocaine are easily washed out from this tissue, it is difficult to remove cinchocaine. Procaine and lignocaine in equal concentrations ($2{\cdot}5 \times 10^{-5}$) also inhibit the stimulant action of benzoylcholine (2×10^{-6}). Local anæsthetic drugs also antagonise the stimulant

TABLE I

FROG RECTUS ABDOMINIS MUSCLE

RELATIVE POTENCY OF LOCAL ANÆSTHETIC DRUGS (PROCAINE = 1) IN ANTAGONISING ACTIONS OF VARIOUS STIMULANTS

Drug	Stimulant			Relative local anæsthetic potency (Elio[3])
	Acetyl choline (2×10^{-7})	Succinyl choline (10^{-7})	Potassium chloride (10^{-3})	
Procaine	1	1	1	1
Lignocaine	0·5	1	1	1·2*
Cocaine	4·2	4·1	4·2	7·4
Cinchocaine	10	10·4	8	10
Concentration of procaine to produce 50 per cent. antagonism	$2{\cdot}5 \times 10^{-5}$	$2{\cdot}5 \times 10^{-5}$	10^{-4}	*Data of Doubleday[5]

action of potassium chloride on the frog rectus muscle but the concentrations needed are about 4 times those required to antagonise the acetylcholine response (Table I).

Higher concentrations of the local anæsthetic drugs (10^{-3}) produce contraction of the rectus muscle, but whereas the stimulant actions of procaine, lignocaine and cocaine are repeatable, that of cinchocaine is not. Tetraethyl pyrophosphate potentiates the stimulant action of acetylcholine 10 to 20 times but has no action on that of the local anæsthetic drugs. Similarly, tubocurarine has little or no effect on the stimulant action of the local anæsthetic drugs.

Isolated rabbit ileum

The contractions produced by acetylcholine are inhibited by all the local anæsthetic drugs. Procaine, lignocaine, cocaine and amethocaine however are equally potent, whilst amylocaine is twice, and cinchocaine 5 times, as potent as procaine (Table II), results which are in line with those reported by Elio[3] using rabbit duodenum. The local anæsthetic drugs inhibit the peristaltic reflex and the nicotine-induced contractions of the rabbit ileum, and in these actions there is better agreement between their relative activities and local anæsthetic potencies than when acetylcholine is the stimulant (Table II). With the exception of procaine, the concentrations required to antagonise these latter responses are ineffective against the acetylcholine response. The local anæsthetic drugs also inhibit the contractions induced by potassium chloride (Table II). Low concentrations of lignocaine and cocaine (and sometimes procaine) increase the normal pendular movements of the rabbit ileum, making quantitative measurements often difficult.

TABLE II

RABBIT ILEUM

RELATIVE POTENCY OF LOCAL ANÆSTHETIC DRUGS (PROCAINE = 1) IN ANTAGONISING ACTIONS OF VARIOUS STIMULANTS AND THE PERISTALIC REFLEX

Drug	Stimulant			Peristalic reflex	Relative local anæsthetic potency
	Acetyl choline (3×10^{-8})	Potassium chloride (10^{-3})	Nicotine (10^{-6})		
Procaine	1	1	1	1	1
Lignocaine	1	1·6	1·4	1·2	1·2
Cocaine	1	2·5	2·4	2·5	7·4
Amylocaine	2·2	10	2·2	2·8	8
Amethocaine	1	10·5	12·5	12·5	8
Cinchocaine	5·1	12·5	20	25·6	10
Concentration of procaine to produce 50 per cent. antagonism	10^{-5}	5×10^{-5}	5×10^{-6}	5×10^{-6}	—

Guinea-pig ileum

In this preparation, procaine regularly produces inhibition of the contraction induced by acetylcholine but the other local anæsthetics often produce variable inhibition. Cocaine usually has about one ninth and lignocaine one fourth the activity of procaine in this respect. Amylocaine is equally potent, whereas amethocaine and cinchocaine are slightly more active than procaine (Table III). Smaller concentrations of the local anæsthetic drugs are needed to inhibit the peristaltic reflex and the contraction induced by nicotine, and there is fair agreement between their relative activities based on these antagonisms and local anæsthetic potencies. When potassium chloride is the stimulant drug, still better agreement is obtained, although higher concentrations of the local anæsthetic drugs are necessary.

Recently, Euler[9] has shown that many nerves contain histamine, and this substance may play some part in nervous conduction. Since the antihistamine drugs possess local anæsthetic activity, it was of importance to study the antihistamine action of local anæsthetic drugs. The dose of procaine needed to inhibit the histamine-induced contraction of the guinea-pig ileum is twice that needed to produce a similar inhibition of the acetylcholine contraction. Cocaine has half, and lignocaine about one sixth, of the activity of procaine in this test; on the other hand, amylocaine, amethocaine and cinchocaine are more active than procaine (Table III).

Isolated cat tracheal chain preparation

The trachea of the cat, like that of the rabbit, is sensitive to acetylcholine but insensitive to histamine (Akcasu[7]). Procaine is a very effective antagonist to acetylcholine, being the most active of the local anæsthetic drugs tested (Table IV). Non-effective doses of lignocaine and cocaine even potentiate the acetylcholine response.

The concentration of procaine which reduces the acetylcholine response does not affect the potassium chloride contraction, and it has to be

TABLE III

GUINEA-PIG ILEUM

RELATIVE POTENCY OF LOCAL ANÆSTHETIC DRUGS (PROCAINE = 1) IN ANTAGONISING ACTIONS OF VARIOUS STIMULANTS AND THE PERISTALTIC REFLEX

Drug	Stimulant				Peristaltic reflex	Relative local anæsthetic potency
	Acetyl choline (4×10^{-9})	Potassium chloride (10^{-3})	Histamine (4×10^{-6})	Nicotine (10^{-6})		
Procaine	1	1	1	1	1	1
Lignocaine	0·2	2	0·2	0·6	0·6	1·2
Cocaine	0·1	2·6	0·5	1	1	7·4
Amylocaine	1	4	2·8	2	2·2	8
Amethocaine	1·2	5·4	3·5	5·4	5·4	8
Cinchocaine	1·6	10·2	4	8·9	6·6	10
Concentration of procaine to produce 50 per cent. antagonism	5×10^{-6}	4×10^{-5}	10^{-5}	10^{-6}	2×10^{-6}	—

TABLE IV

CAT TRACHEA PREPARATION

RELATIVE POTENCY OF LOCAL ANÆSTHETIC DRUGS (PROCAINE = 1) IN ANTAGONISING STIMULANT ACTIONS OF ACETYLCHOLINE AND POTASSIUM CHLORIDE

Drug	Stimulant		Relative local anæsthetic potency
	Acetylcholine (10^{-8})	Potassium chloride (10^{-3})	
Procaine	1	1	1
Lignocaine	0·1	1·3	1·2
Cocaine	0·1	4	7·4
Amylocaine	0·2	5·4	8
Amethocaine	0·3	6·1	8
Cinchocaine	0·2	10·3	10
Concentration of procaine to produce 50 per cent. antagonism	10^{-5}	2×10^{-4}	—

increased 20 to 40 times to give a similar inhibition. However, the same concentrations of the other local anæsthetic drugs are usually equally effective against both acetylcholine and potassium chloride, so that there is good agreement between their relative activities based on the antagonism of the potassium response and their local anæsthetic potencies.

Similar results to those found on the cat trachea have also been obtained on the isolated tracheal chain of the guinea-pig and the isolated human bronchus.

DISCUSSION

Local anæsthetic drugs have been shown to possess at least 3 actions on the frog rectus abdominis muscle. Firstly, they antagonise the nicotinic action of various choline derivatives, and there is good agreement between their relative potencies based on this property and their local anæsthetic potencies. This confirms the results of Elio[3] using acetylcholine as the stimulant drug. Secondly, in somewhat higher concentrations, they antagonise the stimulation produced by potassium

chloride, and again there is good agreement between their relative potencies and their local anæsthetic activities. Such an antagonism has already been shown on the sartorius muscle of the toad (Guarino[10]) and the rectus abdominis muscle of the frog (Blavier, Lecomte, Osterrieth and Vanremoortere[11]), although no quantitative studies were undertaken by these workers. Thirdly, in higher concentrations still, they stimulate the muscle (Zipf[12]) and tetraethyl pyrophosphate and tubocurarine are without effect on this stimulation. As Fleckenstein, Wagner and Googel[13] have already indicated, it may be due to depolarization of the muscle fibres.

Elio[3] stressed the similarity in action between the local anæsthetic drugs and atropine, but a detailed comparison of the atropine-like property of these drugs indicates that in both rabbit and guinea-pig ileum preparations there is little correlation between these actions and the relative local anæsthetic potencies. Similarly, there is no correlation between the antihistamine and local anæsthetic potencies, a fact already reported for the antihistamine drugs by Halpern, Perrin and Dews[14] and Reuse[15].

The relative activities of the local anæsthetic drugs, based on their ability to reduce nicotine response and the peristaltic reflex in the gut agree well with their local anæsthetic activities. Whereas the concentrations of procaine which are effective against the contractions produced by acetylcholine and by nicotine in the gut do not differ widely, those of other local anæsthetic drugs for antagonising the nicotine response are much lower. Greeff[16] has recently reported similar results using procaine and cocaine. The activities of local anæsthetic drugs in reducing potassium chloride responses on the gut are also in line with their local anæsthetic potencies. This may be surprising since in the rabbit ileum potassium chloride produces contraction mainly by ganglionic action (Feldberg[17]). This result supports the hypothesis of Fleckenstein[18,19] that these substances are "anelectronic" and antagonise the responses of catelectronic drugs (e.g. potassium chloride).

The results on the trachea preparations are comparable with those found on the gut, so that there is better agreement between the ability of the local anæsthetic drugs to reduce the potassium chloride response and their relative local anæsthetic potencies than with their ability to reduce that of acetylcholine. Whereas the concentrations of lignocaine, cocaine, amylocaine, amethocaine and cinchocaine are almost the same for both the antagonisms, those of procaine differ widely. For example, the dose of procaine needed to reduce the acetylcholine response has to be increased 20 to 40 times to produce a similar reduction of the potassium chloride response. Nicotine cannot be used as a stimulant in this preparation since it is not effective (Akcasu[7]).

Summary

1. On the frog rectus abdominis muscle, local anæsthetic drugs antagonise the actions of acetylcholine, succinylcholine and potassium chloride. Relative activities based on these properties and their local anæsthetic potencies follow the same trend.

2. The stimulant action of high concentrations of local anæsthetic drugs on the frog rectus muscle has been confirmed. It is little affected by tetraethyl pyrophosphate and tubocurarine.

3. Local anæsthetic drugs antagonise the responses of nicotine and potassium chloride on the rabbit and guinea-pig ileum and abolish the peristaltic reflex. They also antagonise the potassium chloride response on the cat trachea. There is good agreement between their relative activities based on these antagonisms and their local anæsthetic potencies.

4. The atropine-like and antihistamine activities of local anæsthetic drugs on smooth muscle preparations appear to be unrelated to their local anæsthetic potencies.

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