SOME PHARMACOLOGICAL ACTIONS OF CHAKSINE CHLORIDE AND ISOCHAKSINE

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(Received October 8, 1962)

The pharmacological actions of chaksine and isochaksine have been investigated on human beings and on animals, both on intact and isolated tissues. Both the drugs possess a local anaesthetic effect only intradermally. On the guinea pig skin both are inferior to procaine. Procaine is 3.6 ± 1.7 (standard error) times and 1.7 ± 1 (standard error) times more active than chaksine chloride and isochaksine, respectively. Both these drugs produce histamine-like symptoms: itching, erythema, etc. induced by intradermal injection into human skin which can be blocked by giving a parenteral injection of an antihistamine, thus establishing their histamine releasing properties. They also produce hypotension and their mode of action has been discussed. Their neuromuscular blocking properties, on an isolated rat diaphragm preparation, compared with Flaxedil show that Flaxedil is 2 ± 0.2 (standard error) times more potent than chaksine and 4 ± 2.1 (standard error) times more potent than isochaksine, respectively, as a neuromuscular blocking agent. Further clinical trials are also indicated.

Introduction

Cassia absus Linn. grows in different parts of the Indo-Pakistan sub-continent. It is referred to in the vernacular by different names such as 'Chashmizaj' in Arabic, 'Chaksu' in Urdu, and 'Chakhsoo' in Pushto. Various parts of this plant have been used for different ailments. The powdered seeds are largely used as household remedy for ophthalmic diseases. Siddiqui and Ahmad[1] have isolated two water-soluble quarternary bases, namely chaksine (molecular formula, $C_{11}H_{21}O_2N_3$) and isochaksine, the latter being an isomer of the former. The pharmacological properties of this plant have been discussed by Chopra.[2] Mazhar-ul-Haq[3,4] has also described the various actions of chaksine and especially its actions resembling those of Belladona group of drugs. Recently Hye and Wahid[5] have also reported on some actions of chaksine. We felt interested in this problem, as isochaksine was made available to us and specially so, when the actions of chaksine were not fully established. We decided to investigate the pharmacological properties of chaksine and isochaksine, e.g., as local anaesthetic, hypotensive and neuromuscular blocking actions.

Methods

Drugs Used.—Chaksine chloride and isochaksine; Piriton (chlorpheniramine maleate), an antihistaminic compound, kindly supplied by Glaxo Laboratories (Pakistan) Ltd.; acetylcholine, histamine acid phosphate, Flaxedil, morphine sulphate, and *d*-tubocurarine chloride, procaine hydrochloride and cocaine hydrochloride.

Preparations

(1) *Local Anaesthesia.*—(a) Guinea pig's cornea for surface anaesthesia. (b) Guinea pig's skin for infiltration anaesthesia. (c) Human arm intradermal test for infiltration anaesthesia, and the associated local reactions. Controlled human arm intradermal test to note the ability of an antihistaminic to block histamine-like effects.

(2) *Hypotensive Actions.*—(i) Dog's blood pressure and respiration preparation. (ii) Rabbit's blood pressure and respiration preparation.

(3) *Neuromuscular Blocking Property.*—Rat's phrenic nerve diaphragm preparation.

Experimental Procedures

1. Local Anaesthetic Effects

(*a*) *Surface Anaesthesia.*—Cocaine hydrochloride, chaksine chloride and isochaksine were dissolved in 0.9% (w/v) sodium chloride solution. Each drug was constantly applied to the guinea pig's cornea after eliciting the conjunctival reflex by touching it with a horse hair. Concentrations of each drug applied were in the order of 0.1, 0.5, and 1%. The conjunctival reflex was again elicited at intervals of one minute from the time of application of the drug till half an hour. During this time the eyes were inspected for any change in the size of the pupil, light reflex and lachrymation etc.

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(*b*) *Infiltration Anaesthesia on Guinea Pig's Skin.*—Eight guinea pigs (weighing 400-600 g.) were depilated at two places on the back, rear and front, one day before the actual experiment. Two concentrations of both the drugs, one the standard (procaine hydrochloride, the drug commonly used for such purpose), and the other a test drug (chaksine or isochaksine) were injected intradermally at a fixed volume of 0.2 cc. at the above mentioned parts. The doses were so arranged that each concentration was injected synchronically in the front as well as at the back of the animal. The degree of anaesthesia was noted by pricking each part 6 times every 5 minutes both inside the area where the drug was injected as well as on the adjoining area. The normal skin reflex was elicited by noting the presence of twitching after pin prick. This experiment was continued for 1/2 hour. The total number of failures in twitching to pricks was counted as the degree of anaesthesia. The results were obtained by plotting the concentration of the drug in doses as abscissae against the degree of anaesthesia as ordinates. The greater the anaesthesia the larger the number of missed pricks (as shown in Fig. 1).

Procaine hydrochloride:
concentrations used 0.0125% and 0.025%
Chaksine chloride and isochaksine:
concentrations used 0.025% and 0.1%

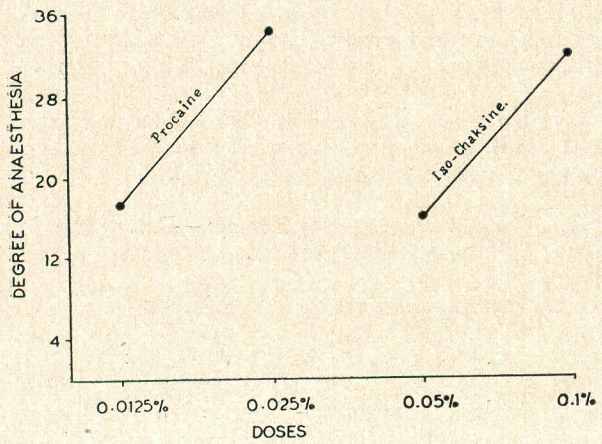

Fig. 1.—Guinea pig's intradermal test. The result of a single four-point assay, comparing the local anaesthetic effects of procaine with that of isochaksine. Note that a smaller concentration of procaine solution has produced anaesthesia comparable to a higher concentration of isochaksine.

(*c*) *Infiltration Anaesthesia on Human Arms.*—Availing the experimental pharmacology student classes at the Khyber Medical College, Peshawar University, and also utilizing the services of the volunteers amongst the laboratory staff at the North Regional Laboratories, both chaksine chloride and isochaksine were injected intradermally in 0.5 cc. volume of a 1% solution, dissolved in normal saline solution, into human forearms on the flexor surface.

The degree of anaesthesia was compared with that of cocaine hydrochloride (1%) and procaine hydrochloride (1%), with and without adrenaline, and normal saline solution. The presence of anaesthesia was determined by pin-prick sensation. Any other signs, like erythema, and symptoms like itching, if present on the forearm, were also observed.

2. HYPOTENSIVE ACTION

Dog's/Rabbit's Blood Pressure and Respiration Preparation.—Dogs weighing (7-10 kg.) and rabbits weighing (1500-1800 g.) were anaesthetised with pentobarbitone sodium (5% soln.) 35 mg./kg. intraperitoneally and urethane 25% solution 10 cc./kg. intravenously, respectively. The carotid blood pressure and respiration of the animal was recorded with a mercury manometer, using Gaddum's tambour, on a smoked drum.

Average doses of drugs given intravenously are as under:—

Rabbits: (1.5 to 1.8 kg.)

Histamine	5-10 µg.
Acetylcholine	2-5 µg.
Chaksine and isochaksine	2-3 mg.
Piriton	5 mg.

Dogs: (7-10 kg.)

Histamine	10-30 µg.
Acetylcholine	5-10 µg.
Chaksine chloride and isochaksine	5-10 mg.
Piriton	5-10 mg.
Morphine sulphate	50-75 µg.
d-Tubocurarine	1-3 mg.

Histamine and acetyl choline were injected to plot the dose-response curves against their hypotensive effect. Isochaksine was also injected to note its effect on this preparation, which showed a fall in blood pressure. The hypotensive effect of chaksine also was studied for further clarification.

During this study it was found that both chaksine and isochaksine, after intradermal injection, produced local signs and symptoms similar to histamine on human arms, and that these symptoms could be relieved by giving a parenteral dose

of an antihistaminic drug. Thus we presumed that the hypotensive effect of both chaksine and isochaksine may also be due to the release of histamine which may be antagonised by a parenteral injection of antihistaminic drugs. We arranged experiments to test this hypothesis.

3. Neuromuscular Blocking Property

An albino rat weighing about 200 g. was killed and its diaphragm on one side alongwith its phrenic nerve was isolated and suspended in Tyrode's solution in a 75-ml. bath at 37 °C., as described by Bulbring.[6] The muscle was indirectly stimulated through the nerve by applying a stimulus varying from 30 to 50 milliamperes for an interval of 1 to 5 milliseconds. The contractions were recorded on a smoked drum in a cycle of 5-minute period. A three-point assay was performed, using Flaxedil (5-10 mg.) as a standard drug and chaksine (5-15 mg.) or isochaksine (10-30 mg.) and their relative potencies in terms of Flaxedil were found by matching.

Results

1. Anaesthetic Effects

(*a*) *Surface Anaesthesia.*—We did not find any evidence of surface anaesthesia or any other change in the guinea pig's eyes by concentrations even up to 1.0% of chaksine chloride and isochaksine when applied for an hour, while in the control eyes 0.1% cocaine hydrochloride produced marked surface anaesthesia. Hye and Wahid[5] also reported that varying concentrations of chaksine when put into rabbit's cornea failed to produce surface anaesthesia.

(*b*) *Infiltration Anaesthesia on Guinea Pig's Skin.*—Four-point assays comparing the potencies of chaksine and isochaksine against procaine hydrochloride were carried out. These tests showed that procaine hydrochloride is 3.6 ± 1.7 (standard error) times as active as chaksine chloride. Similarly procaine hydrochloride was found to be 1.7 ± 1 (standard error) times more potent than isochaksine (Fig. 1). Both the results are concluded from three sets of experiments. Thus isochaksine is about two times more active than chaksine but both are less active than procaine. Hye and Wahid[5] also reported that chaksine has some local anaesthetic activity.

(*c*) *Infiltration Anaesthesia on Human Arms.*—By injecting chaksine chloride and isochaksine intradermally we noted that both these drugs produced a very feeble anaesthesia, but intense itching, burning and local erythema, at the site of injection

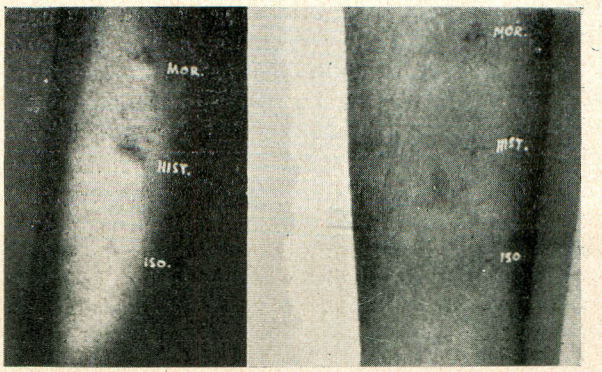

Fig. 2.—Human forearm test. Two forearms of the same individual. The control forearm (on the left) has been injected intradermally, at three places, from above downwards with solutions of morphine sulphate, histamine and isochaksine. All the three drugs produce indurations of varying sizes.

The test forearm (on the right) is injected with the same three drugs in the same order but 30 minutes after a parenteral dose of Piriton has been given. Please note that none of the three drugs have produced any induration in this test forearm.

(Fig. 2). This continued for about 20 to 30 minutes while procaine hydrochloride and cocaine produced definite anaesthesia (with no other symptoms) with immediate onset and lasting for over half an hour. The injected arms when inspected for the next two days were found to be normal. These local reactions to chaksine chloride and isochaksine were similar to the one produced by injecting intradermally 0.2 cc. of a 2% solution of histamine or on scratching over a drop of histamine or a histamine liberator such as morphine sulphate (Fig. 2). If, however, an intramuscular injection of any antihistaminic is given to the human subjects, on whom one has demonstrated local effects of histamine, chaksine and isochaksine or any of the classical histamine liberators, e.g., morphine sulphate, it will become evident that neither histamine nor chaksine, isochaksine and the histamine liberators produce their local effects (Fig. 2). This is a convincing evidence in favour of the fact that local reactions, e.g., erythema and itching etc., to the intradermal chaksine and isochaksine, are due to the local release of histamine.

2. Hypotensive Action

Dog's/Rabbit's Blood Pressure and Respiration Preparation.—Chaksine chloride and isochaksine (2 mg. to 10 mg.) produced appreciable fall in blood pressure comparable to that by histamine (5 to 30 µg.) and acetylcholine (2 to 10 µg.). The onset of the fall in blood pressure to chaksine and isochaksine was delayed by about 10 to 20 seconds, while after the injection of histamine and acetylcholine it was instantaneous. The fall due to chaksine and isochaksine was more sustained than

that with histamine or acetylchloine and was not blocked by atropine. After injecting Piriton, in doses of 5-10 mg. intravenously to the animal, after getting equiactive responses to acetylcholine, histamine, chaksine and isochaksine, we noted that Piriton invariably blocks the responses to histamine but it did not significantly effect the responses to acetylcholine, chaksine and isochaksine (Fig. 3). In two experiments, however, the responses to isochaksine were slightly reduced by Piriton. The results in rabbits were similar to dog's blood pressure preparation. As seen from human arm experiments that like chaksine and isochaksine, morphine, a liberator of histamine, also produced local symptoms similar to histamine which could be prevented by a parenteral injection of an antihistamine. We also tried to see whether the hypotensive effect of other histamine liberators, e.g., morphine sulphate (75 μg.) and *d*-tubocurarine (1-3 mg.), in dog's blood pressure preparation can be blocked by Piriton or not. In a few sets of experiments we found that like chaksine and isochaksine the hypotensive effect of morphine, as well as that of *d*-tubocurarine, a

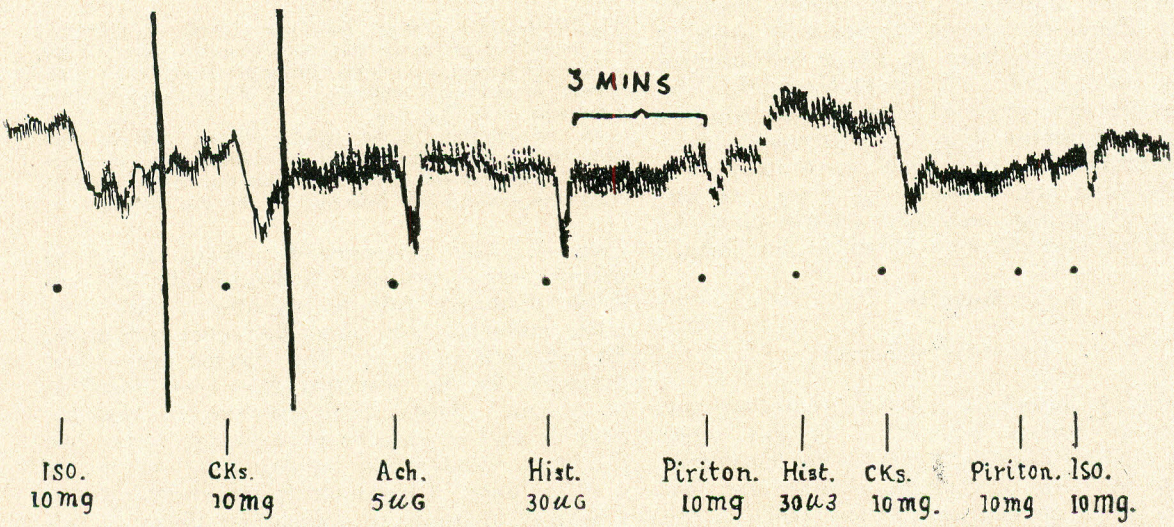

Fig. 3.—Dog's blood pressure preparation. The responses to the various drugs in an anaesthetized dog's blood pressure preparation before and after an intravenous dose of chlorpheniramine maleate (Piriton). Note that the hypotensive effects of chaksine and isochaksine are more prolonged as compared to the similar effects of acetyl choline and histamine. Piriton seems to block the hypotensive effect of histamine and that of isochaksine is reduced by Piriton in this and one more preparation but was not repeated in four other such experiments.

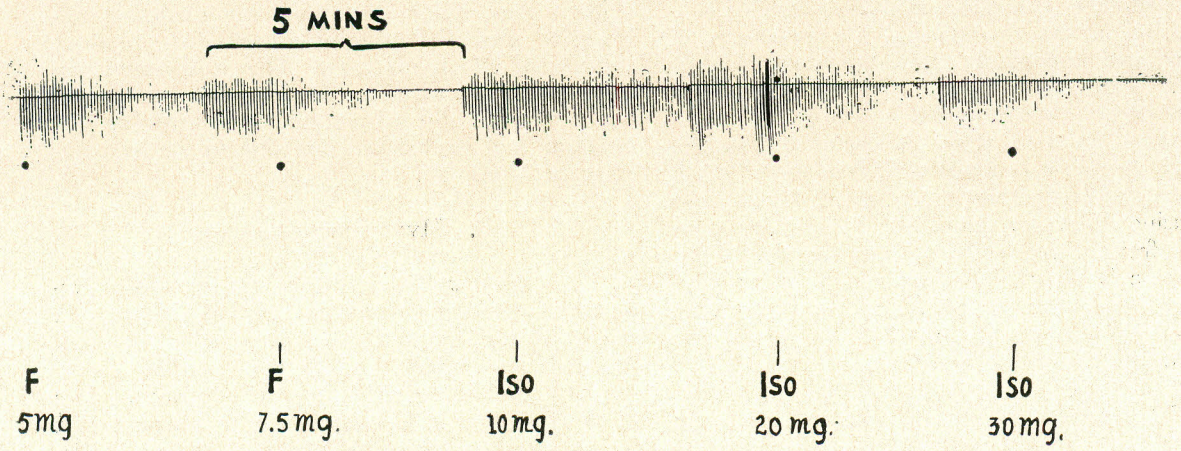

Fig. 4.—Rat phrenic nerve diaphragm preparation. The effect of Flaxedil (F) 5 mg. and 7.5 mg. and that of isochaksine (Iso) 10, 20 and 30 mg. The more potent the dose of a neuromuscular blocking drug, the greater is the reduction in the amplitude of contraction of the diaphragm.

potent histamine releaser, was also not blocked by Piriton.

3. Neuromuscular Blocking Property

Chaksine reduced the contractions of this muscle in about 60 to 90 seconds after the application of the drug, and complete recovery takes place after 3-4 minutes when the drug is washed out. Isochaksine required 3 to 4 minutes to produce a block, while its effect passes off in 4 to 5 minutes after the drug is washed out. Flaxedil produces blocking action in about 60 seconds and recovery after the washing is completed in about 2 minutes time. The results of the three 3-point assays each with Flaxedil and the test drug, chaksine and isochaksine, reveal that Flaxedil is 2 ± 0.2 (standard error) times more potent than chaksine, while Flaxedil is 4 ± 2.1 (standard error) times more potent than isochaksine (Fig. 4).

Discussion

Local Anaesthetic Effect.—It is interesting to note that both drugs show very little, if any, surface anaesthetic effect and thus cannot relieve the symptoms of conjunctivitis as such. Both drugs produce intradermal anaesthesia which in both cases is significantly lower than that of procaine. Their suitability as local anaesthetics in clinical practice is further limited by the production of side effects such as itching, burning and swelling etc. at the site of injection and thus masks its feeble intradermal anaesthetic effect. One can convincingly say that these side effects are due to the release of histamine at the site of injections similar to other histamine liberators because of the similarity of symptoms with those of the histamine application to the skin and by the ability of antihistaminics to prevent these effects. The investigation of these two drugs as well as the crude product for their antibacterial properties may throw more light on the mode of their effectiveness in purulent conjunctivitis for which the crude drug is very popular. It is also interesting to note that local application into guinea pig's eye, produced no dilatation of the pupil and seems devoid of atropine-like actions in the concentrations used.

Hypotensive Effects

There seems to be more than one way for these drugs to produce hypotension in anaesthetised animals.

(i) *Ganglion Blocking Effects.*—Cheema[7] has convincingly shown that isochaksine possesses a ganglion blocking action. Khan and Bukhari[8] have also observed ganglion blocking (antinicotine) activity in both chaksine and isochaksine by using isolated guinea pig ileum preparations.

(ii) *Central Depression.*—Cheema[7] has also demonstrated that isochaksine produces hypotension partly by its central action by using spinal cats.

(iii) *Histamine Release.*—From our results it is absolutely clear that both chaksine and isochaksine possess the ability to release histamine when injected locally. A drug which releases histamine when injected locally can do so even if it is given intravenously; *d*-tubocurarine chloride which is primarily a neuromuscular blocking agent and also a histamine releaser, can sometimes produce untoward symptoms similar to a parenteral dose of histamine, where hypotension is a marked feature. The question arises whether hypotension produced by a parenteral dose of a drug, established to be a histamine releaser, can be prevented or antagonised by the parenteral treatment with an antihistamine. We have shown in our results that hypotension produced by intravenous injections of the two potent histamine releasers, morphine sulphate and *d*-tubocurarine, is resistant to earlier treatment by an active antihistamine. Thus the inability of the same antihistaminic to reduce the hypotensive effects of chaksine and isochaksine in similar preparations, cannot exclude the hypothesis that both these drugs produce hypotension partly due to their ability to release histamine. We now propose to use dogs where the histamine store of their bodies is being depleted by treatment with compound 48/80 and repeat these experiments. It will also be interesting to note the actions of these two drugs, chaksine and isochaksine, in animals and human beings when given orally.

Neuromuscular Blocking Property

Both these drugs do not seem to have enough neuromuscular blocking effect to encourage us to arrange for clinical trials for such an action.

Acknowledgements.—Our thanks are due to Dr. S.A. Warsi, Director, North Regional Laboratories, for his keen interest and encouragement, and to the staff of the Department of Pharmacology, Khyber Medical College, Peshawar, for helping us in this work.

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