THE PHARMACOLOGY OF CHAKSINE CHLORIDE

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Chaksine chloride, the alkaloid from *Cassia absus*, was prepared in a pure form by known methods. Its pharmacology was tested on experimental animals and isolated organs. Its neuromuscular blocking activity was specially investigated, and it was found to have marked curare-like activity. Its anti-cholinergic action on smooth muscles and its effects on blood pressure and respiration are also discussed. Toxicity of chaksine subcutaneously in mice was determined.

Chaksine is the alkaloid from the seeds of *Cassia absus* and was first isolated as carbonate by Siddiqui and Ahmed.[1] It was assigned the formula $C_{12}H_{21}O_2N_3$ and was described as a quaternary base, forming salts $C_{12}H_{20}ON_3X$ by loss of water. Later Kapur, Gaind, Narang and Ray[2] suggested the new empirical formula $C_{11}H_{21}O_3N_3$ but Puri, Sharma and Siddiqui[3] found that the analytical results with benzoyl benzoate supported the C_{12} formula, although those for organic salts were in better agreement with C_{11} formula.

Mazhar-ul-Haque[4] reported some pharmacological properties of chaksine sulphate. Chaksine sulphate is only sparingly soluble in water or in hot alcohol, but the chloride is soluble in water. Chaksine chloride was prepared and investigated for its pharmacological properties.

Chaksine iodide (m.p. 168°C. dec.) was first prepared in the usual manner and was allowed to react with a suspension of freshly prepared neutral silver chloride, whereby chaksine chloride separated out as cyrstalline precipitate. The salt was further purified and recrystallized from a mixture of alcohol and acetone. The final product was white, crystalline (needle-shaped) and soluble in water and had a melting point 178°C. The purity of the product was also tested by chromatography and paper electrophoresis.

Pharmacological

Methods and Material

Composition of Perfusion Fluid (*g./litre*).—Frog ringer's solution: NaCl 6.5, KCl 0.14, $NaHCO_3$ 0.20, $CaCl_2$ 0.12, Dextrose 1.0. Tyrode's solution: NaCl 8.0, $NaHCO_3$ 1.0, KCl 0.2, $CaCl_2$ 0.2, $MgCl_2$ 0.1, NaH_2PO_4 0.05, Dextrose 1.0 Locke's solution: NaCl 9.0, $NaHCO_3$ 0.15, KCl 0.42, $CaCl_2$ 0.24, Dextrose 1.0.

Drugs Used.—Chaksine chloride, acetylcholine chloride, adrenaline hydrochloride, hexamethonium bromide, neostigmine methyl sulphate (Prostigmin), gallamine triethiodide (Flaxidil), histamine acid phosphate.

Neuromuscular Blocking Activity

Frog Rectus Abdominis Muscle.—Reproducible submaximal contractions of the rectus muscle were induced by acetylcholine chloride, in a 12 ml. bath at room temperature. Acetylcholine chloride was left in contact with the muscle for 1.5 minutes and doses were repeated at 5-minute intervals. Chaksine chloride was added 0.5 minute before the addition of acetylcholine chloride, and was thus in contact with the muscle for two minutes for each dose.

Rabbit Head-drop Method.—Rabbits of either sex, weighing 1.5 to 2.2 kg., were used. Solution of chaksine chloride 1.0 mg./ml. in normal saline was administered by infusion into one of the ear veins at a rate of 1 ml./min. Infusion continued until, following a light tap on the muzzle, the animal failed to raise its head. Six animals were used to find the head drop dose.

Rat Diaphragm-Phrenic Nerve Preparation.—Method described by Bulbring[6] and others[7] was used for estimating curariform activity. A platinum electrode was used and the muscle stimulated indirectly. Six square wave stimulations of 1 millisecond duration were applied per minute at 8 volts. The bath was 100 ml. oxygenated Tyrode solution at 37 °C. Drugs were left in contact for 3 minutes before washing and successive doses were given at 10 minutes intervals.

Other Properties

Effects on Blood Pressure and Respiration. — Cats and rabbits under chloralose anaesthesia (90 mg./kg. intraperitoneally) were used. All drugs were administered by injection, into the femoral vein. Blood pressure was recorded from the carotid artery and respiration by tracheal canula, and a Mary's tambour. Five hundred i.u./kg. of heparin was injected into the vein and after dissection was completed another 500 i.u. was placed inside the canula. Drugs were injected at 10 min. intervals. Blood pressure and respiration was also recorded in rabbits under continuous infusion of chaksine chloride with a slow injector.

Effects on Isolated Rabbit Hearts.—Rabbit hearts were perfused by Langendorff's method,[8] with oxygenated Locke's solution at 37°C. Outflow was recorded by collecting perfusate on a Condon's outflow recorder (magnet-tipper). Drugs were injected through canula directly at the point of attachment of the aorta.

Rabbit Duodenum.—Pieces of duodenum 5-6 cm. long were suspended in a 50 ml. bath containing oxygenated Tyrode's solution at 37°C. The spontaneous movements of duodenum were recorded. Contractions with acetylcholine chloride were allowed to remain for 30 sec.

Guinea Pig Ileum.—Pieces of the terminal ileum about 4 cm. long were suspended in oxygenated Tyrode's solution at 32°C. Acetylcholine chloride was allowed to remain in contact for 30 seconds and doses were added at 3 to 4-minute intervals.

Isolated Rabbit Auricle.—The isolated auricles were set up[5] in a 60 ml. bath of oxygenated Ringer Locke solution at 29°C. and contractions were recorded through a light straw.

Local Anaesthetic Activity and the Effect on Pupillary Muscles.—Local anaesthetic activity was investigated by Bulbring and Wajda[9] method by intradermal local injections on a group of guinea pigs and was compared with the effect of cocaine hydrochloride. Chaksine chloride at different concentrations were applied on rabbit's cornea for observing local action on the eyes. It was also given subcutaneously in rats and the size of the pupils were observed, under a dissecting microscope.

Toxicity Determinations.—Chaksine was injected subcutaneously in 42 white mice in 7 groups of 6 mice. Percentage of mortalities were converted to working probits and LD_{50} calculated graphically and also statistically.

Results

Frog Rectus Abdominis Muscle.—Chaksine chloride antagonised the action of acetylcholine chloride on the frog rectus. Two μg./ml. of chaksine chloride reduced the contractions induced by 0.25 μg./ml. of acetylcholine chloride to 50 per cent. Gallamine, 0.4 μg./ml. produced similar effects. The effect of chaksine was completely reversible after 2-3 washings. But several more washings were required when the doses of chaksine were more than 6 μg./ml. Doses of chaksine less than 0.2 μg./ml. had no perceptible effect and a dose of 10 μg./ml. reduced acetylcholine chloride contractions (0.25 μg./ml.) almost to nil. The dose response curve of chaksine on this preparation shows a linear regularity. Figure 1 shows the effect of chaksine chloride on frog rectus abdominis muscle preparation.

Rat Diaphragm - Phrenic Nerve.—20 μg./ml. of chaksine chloride just reduced the contractions perceptibly. 30 μg./ml. reduced twitch height by 35-40 per cent. The effects were reversible but a

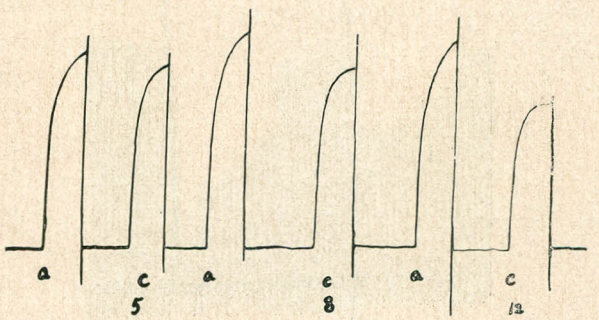


Fig. 1(a)

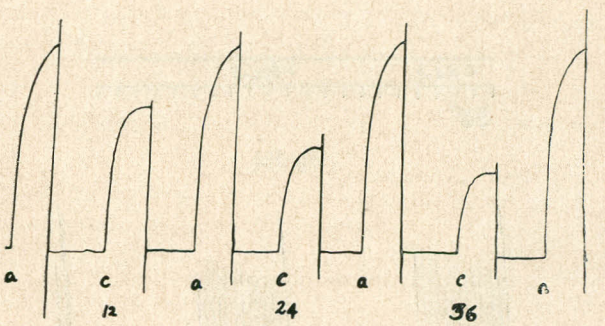


Fig. 1(b)

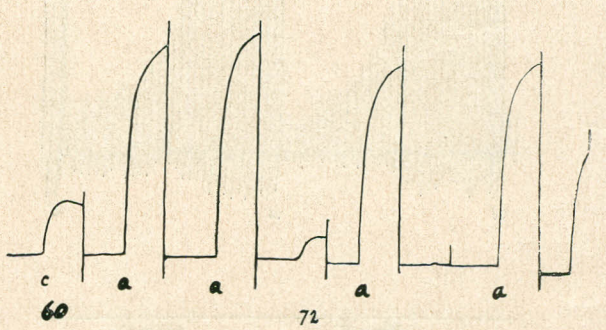


Fig. 1(c)

Figs. 1(a,b,c).—The effect of chaksine on the isolated frog rectus abdominis muscle. Bath volume 12 ml. Contractions labelled 'a' are due to 3 μg. of acetylcholine (0.25 μg./ml.) for 1.5 minute. Contractions labelled 'c' are due to the same dose of acetylcholine for the same time but each addition was preceded 0.5 minute earlier by addition of chaksine in the bath. The numbers below 'c' indicate μg. of chaksine added.

complete block was not produced with 200 μg./ml. of chaksine. On this preparation chaksine proved to be about half as effective as gallamine. Figure 2 shows the effect of chaksine on this preparation.

Rabbit Head Drop.—The average dose to cause head drop in a group of 6 rabbits (1.5 to 2.2 kg.) was 5.8 mg./kg. One of the animals died shortly after the onset of head drop. In others head drop lasted for 15-20 minutes but the animals were not capable of normal movements for more than two hours after the injections and during this period assumed a characteristic posture.

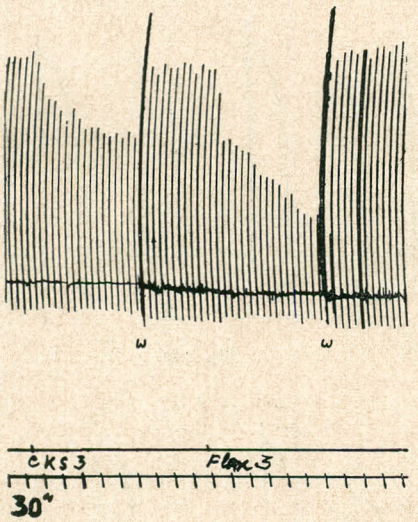


Fig. 2(a)

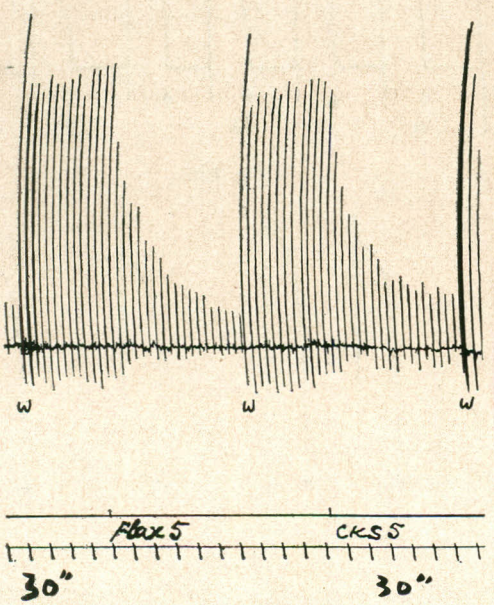


Fig. 2(b)

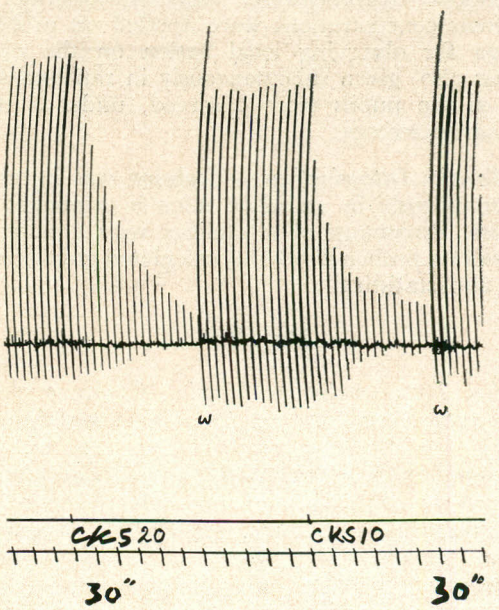


Fig. 2(c)

Figs. 2(a,b,c).—Rat phrenic nerve-diaphragm preparation. Contractions are due to indirect square pulses, 6 per minute at 8 volts and of 1 millisecond duration. (a) 30 μg./ml. of chaksine and 30/μg. ml. of gallamine. (b) 50 μg./ml. of gallamine and 50 μg./ml. of chaksine. (c) 200 μg./ml. of chaksine and 100 μg./ml. of chaksine.

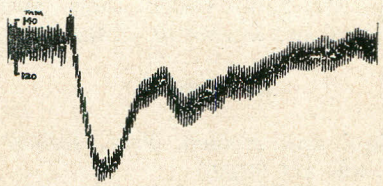
Other Properties

Blood Pressure and Respiration.—In chloralosed cats 0.6 mg./kg. of chaksine caused a marked fall in blood pressure (30-40 mm. Hg.) along with a fall in respiratory volume (Fig. 3). The rate of respiration was not changed. Both blood pressure and respiration returned to normal after about 5 minutes. With 1.3 mg./kg., blood pressure fell rapidly by 50 to 60 mm. of Hg. and respiration was much depressed and slowed, showing the signs of failure. The blood pressure recovered after 8-10 minutes but the respiration was irregular. Neither antagonism nor potentiation was caused by chaksine chloride to the characteristic actions of acetylcholine chloride (0.5-1.0 μg./kg.) or adrenaline hydrochloride.

The lowering effect of chaksine chloride on blood pressure was obtainable even after 1 mg./kg. of hexamethonium bromide or 2 mg. of atropine.

Continuous infusion of chaksine chloride caused respiratory failure but artificial respiration restored the animals. In two rabbits under urethane anaesthesia 1 mg./ml. solution of chaksine chloride was injected at the rate of 0.5 ml./min. (i.e. 0.5 mg./min.) using a continuous slow injector.

Chaksine chloride at first caused irregularity of the respiration and it was followed by slowing of the rate. After 10-11 mg./kg. of chaksine chloride respiration became shallow, and it stopped completely after 19.4 and 21.2 mg./kg. of chaksine chloride had been infused. After artificial respiration with a pump for 5 minutes, the animals recovered and respiration started. Within the next 10 minutes respiration was quite regular and deep.



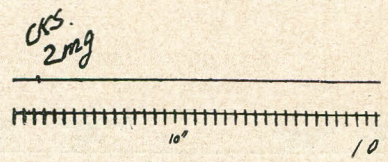


Fig. 3(a)

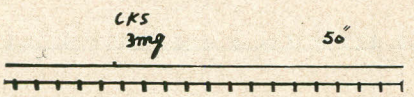


Fig. 3(b)

Fig. 3.—(a) Effect of chaksine on the blood pressure of cat (3.02 kg.) anaesthesia by chloralose 90 mg./kg. intraparetoneally). Chaksine was given by intravenous injection.

(b) Effect of chaksine on the blood pressure and respiration of rabbit (1.5 kg., anaesthesia by chloralose 90 mg./kg. intraparetoneally).

Isolated Rabbit Heart.—0.3 to 0.5 mg. of chaksine chloride had no effect on the rate, amplitude or outflow of the isolated rabbit's heart. But doses about this level (0.5-2 mg.) showed a temporary slowing of the heart rate and diminished outflow.

ANTICHOLINERGIC ACTION

Isolated Rabbit Duodenum.—2-10 μg./ml. of chaksine chloride reduced but did not completely paralyse the movement of isolated rabbit duodenum. Contractions induced by acetylcholine chloride (3 μg. in a 50 ml. bath) was markedly reduced or blocked by 100-200 μg. of chaksine chloride. But even 2.5 mg. of chaksine chloride (50 μg./ml.) could not completely block the acetylcholine chloride action. The tissue recovered after washings. Ten μg./ml. of chaksine chloride could completely block the increase of tone obtainable with 0.1 μg./ml. of neostigmine methyl sulphate (Prostigmin). Figure 4 shows effect of chaksine on isolated rabbit duodenum.

Guinea Pig Ileum.—Chaksine chloride shows anticholinergic action on this preparation as well. 0.3 μg./ml. of chaksine chloride reduced acetylcholine chloride-induced (0.1 μg./ml.) contractions by 80 per cent. Doses less than this of chaksine chloride reduced acetylcholine contractions proportionately, but even higher doses (1 μg./ml.) could not block acetylcholine action completely.

Isolated Rabbit Auricle.—On this preparation (60 ml. bath) chaksine chloride by itself had no effect, but it showed anticholinergic action. Reduction of amplitude and slowing was induced by 20 μg. acetylcholine chloride. 0.3 mg. of chaksine chloride restored this reduction to normal (Fig. 5). Alternatively when chaksine chloride dose was followed by that of acetylcholine chloride, the latter failed to cause slowing and reduction of amplitude, as it would have done alone.

Local Anaesthetic Action.—On guinea pig skin by intradermal wheal method, it was observed that chaksine chloride has some local anaesthetic action. Its effects lasted longer than those of cocaine. Chaksine chloride had no surface anaesthetic action on rabbit cornea.

Toxicity in Mice.—In a group of 42 white mice divided into 7 groups, the LD_{50} subcutaneous was found to be 93.6 mg./kg. body weight. The calculation was done statistically from the working probits.

Summary and Conclusion

Pharmacology of chaksine chloride was investigated with special importance to its neuromuscular blocking activities.

From its different actions, chaksine chloride can be considered important pharmacologically. The structure, when determined, may reveal further clues and it may be possible to change it to get a more effective muscle relaxant drug.[11]

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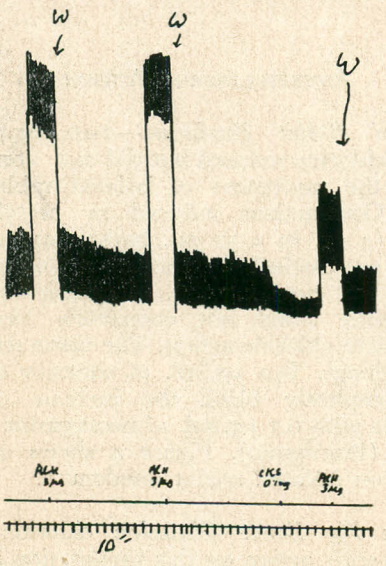

Fig. 4(a)

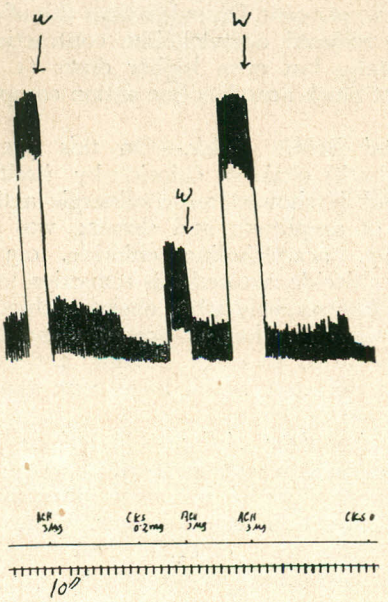

Fig. 4(b)

Fig. 4.—(a,b). Isolated rabbit duodenum; bath 50 ml. Contractions are due to 3 µg. of acetylcholine for 30 seconds. Addition of chaksine relaxes tone and when followed after 1 minute by acetylcholine, shows anticholinergic action.

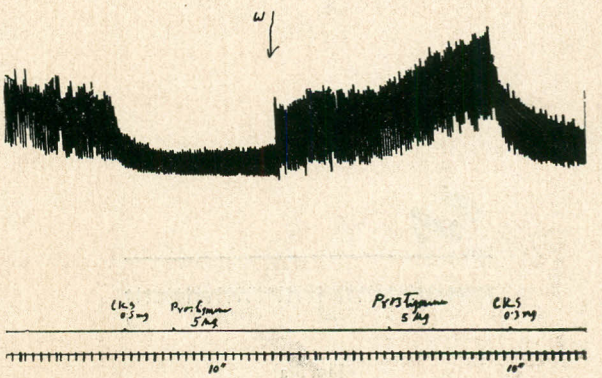

Fig. 4(c)

Fig. 4. (c).—Antagonism of chaksine and neostigmine on isolated rabbit duodenum. Bath 50 ml. A test dose of neostigmine 5 µg. given earlier is not shown in figure. Chaksine dose is 500 µg. (10 µg./ml.) and neostigmine doses are 5 µg. (0.1 µg./ml.).

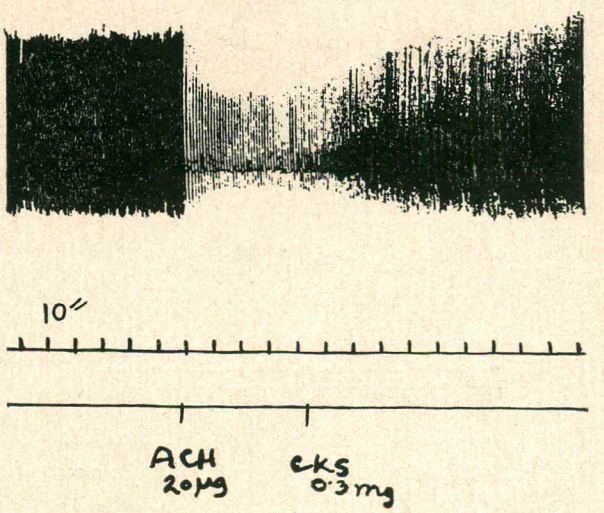

Fig. 5.—Isolated rabbit auricles; bath 60 ml. Dose of 20 µg. acetylcholine chloride followed by 0.3 mg. of chaksine chloride.

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