SYNERGISTIC ACTION OF ISOCHAKSINE AND SERPAJMALINE

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The pharmacological properties and toxicity of isochaksine have been investigated. Isochaksine produced marked hypotension following intravenous injection in dogs. The mechanism of this hypotension was both central as well as peripheral. Isochaksine had an inhibitory action on the cardiac musculature. It also showed ganglion blocking activity. Isochaksine showed a synergistic action with serpajmaline in producing a hypotensive response both in conscious as well as in anaesthetised animals.

Introduction

Isochaksine is an alkaloid isolated by Siddiqui and Ahmed[1] from *Cassia absus* Linn. (chaksu). The chloride salt of this compound is a white, extremely hygroscopic crystalline powder which is easily soluble in water. Whereas the pharmacology of its parent compound chaksine is well known,[2-7] very little pharmacological work has so far been done on isochaksine. In indigenous medicine the seeds of *Cassia absus* are used in the treatment of opthalmia and skin affections.[8]

Serpajmaline is an alkaloidal complex isolated by Siddiqui et al[9] from Rauwolfia serpentina. The pharmacology of this compound has been reported by Deininger[10] and by Child et al.[11] This compound is free from reserpine and has been reported to have a strong hypotensive activity.

The authors have studied the toxicology and pharmacology of isochaksine with special reference to its cardio-vascular properties. The possibility of a synergistic action between isochaksine and serpajmaline has also been explored.

Methods

Toxicity.—Acute toxicity of isochaksine was studied in adult male rats. The animals were divided in two groups and the drug was given by intraperitoneal and subcutaneous routes, and the percentage mortalities were assessed twenty four hours later. LD 50 values were determined by the Probit method.

Isolated Rabbit Heart.—Rabbit hearts were perfused by the Langendroff method. The hearts were perfused with McEwen's solution at 37°C. and at a perfusion pressure of 40 cm. of water. The perfusion fluid was kept fully saturated with oxygen. The contractions of the heart were recorded on a smoked drum. Drugs were added via a polythene cannula placed near the aortic opening of the heart.

Fibrillation was induced in normally beating hearts by electrically stimulating the ventricles via two thin platinum electrodes. After fibrillating for ten minutes the hearts never spontaneously reverted to normal rhythm unless some drug was given.

Blood Pressure.—Dogs of either sex were used for the study of the blood pressure. Anaesthesia was induced by injecting sodium pentothal 15 mg./kg. and sodium gardenal 25 mg./kg. intravenously followed by an injection of sodium gardenal 75 mg./kg. intraperitoneally. Blood pressure was recorded from the cannulated carotid artery and the trachea was cannulated for recording respiration. Drugs were injected in a femoral vein. In certain experiments the blood pressure was recorded from femoral artery. In these animals the carotid arteries were isolated and clamped periodically.

Nictitating Membrane Preparation.—The contraction of the nictitating membrane from anaesthetised cats (sodium gardenal 1 mg./kg. intraperitoneally) was recorded on smoked drum with a sensitive lever.

Preganglionic stimulation of the cervical sympathetic nerve was maintained for 15-seconds period with square wave pulses of 5 to 7 volts at a frequency of 10 per second and a duration of 0.5 msec. Drugs were injected into the cannulated femoral vein while blood pressure was recorded via the contralateral carotid artery.

Blood Pressure of the Conscious Dogs.—Normal dogs were operated upon under anaesthesia and one of their carotid arteries was exteriorised through the muscle layer and was sutured in a fold of skin. When the wound healed completely, the systolic blood pressure was measured by applying a cuff on the loop which compressed the artery. Drugs were injected into the saphenous vein.

Results

Toxicity.—The results are given in Table 1.

Soon after giving lethal doses of isochaksine, the rats seemed to be dull and showed signs of muscular weakness. The respiration was not markedly affected and the death appeared to be due to cardiac toxicity.

The toxicity of serpajmaline in rats has already been reported by Child et al [11]. They have reported the LD 50 as 230 mg./kg. body weight when given intraperitoneally. When isochaksine was combined with serpajmaline in a ratio of about 1:7, the toxicity of both the drugs was modified. Experiments were done in a manner similar to that described before. The results are given in Table 2. It appears from these results that the toxicity of serpajmaline is slightly increased when it is combined with isochaksine.

TABLE 1.—TOXICITY OF ISOCHAKSINE.

Animals	Sex	LD 50 value - mg./kg.	
		Subcutaneous	Intraperitoneal
Rats	Male	154	111

TABLE 2.—TOXICITY OF A COMBINATION OF ISOCHAKSINE AND SERPAJMALINE.

Animals	Sex	LD 50 value - mg./kg.
		Intraperitoneal
Rats	Male	196 (Isochaksine = 24) (Serpajmaline =172)

Isolated Rabbit Heart.—In a dose of 10μg., isochaksine caused a decrease in the amplitude of the heart muscle. The amplitude goes on decreasing after every application until the heart stops. After a single application of 10μg. isochaksine, the amplitude returns to normal after two to three minutes. Isochaksine also reduced the coronary flow. The normal flow of 8 ml./min. was reduced to 4 ml./min. after a dose of 10μg. isochaksine. This action is also short lasting and the flow returns to normal within 4 minutes. In the same doses isochaksine caused definite bradycardia but this effect was also momentary.

Isochaksine in doses upto 300μg. does not show any antifibrillatory activity. After the heart was artificially fibrillated by electrical stimulation, it could not be revived by isochaksine. Serpajmaline 220μg. was, however, successful under similar circumstances.

Blood Pressure.—Isochaksine produced a sharp fall in the blood pressure of both dogs and cats, after an intravenous dose of 0.25 mg./kg. The blood pressure returned to normal after about 15 minutes. A dose of 0.5 mg./kg. produced a much more pronounced fall. The hypotensive responses were not, however, reproduceable in the same animal due to tachyphylaxis. Fig. 2 demonstrates the effect of 0.5 mg./kg. isochaksine on the blood pressure of a dog.

In order to ellucidate the mechanism of hypotensive action of isochaksine certain experiments were done on dogs and cats. Fig. 3 a little mod shows the record of the femoral artery blood pressure of a dog, whose carotid arteries were exposed and occluded by clamps at regular intervals for a short duration. This produced a sharp rise in the blood pressure. Isochaksine in a dose of 0.25 mg./kg. produced moderate lowering of the blood pressure as well as a marked reduction in the carotid occlusion response. It could therefore be concluded that the hypotensive action is at least partly central in origin.

The mechanism of the hypotensive action of isochaksine was further studied in a fresh series of experiments on dogs. As is demonstrated in Fig. 4, 0.25 mg./kg. isochaksine lowered the blood pressure even after procedures like vagotomy, injections of atropine 2 mg./kg. and hexamethonium 5 mg./kg.

Nictitating Membrane Preparation.—Electrical stimulation of the cat sympathetic chain produced a sharp contraction of the nictitating membrane which relaxed completely within one or two minutes. Isochaksine in a dose of 0.25 mg./kg. given intravenously caused a sharp contraction of the nictitating membrane. This was, however, followed by abolition of the responses to preganglionic electrical stimulation. The response to intravenous adrenaline remained, on the other hand, unaffected (Fig. 5).

Synergistic Action between Isochaksine and Serpajmaline.—Serpajmaline produced a marked fall in the blood pressure of the dog when injected in a dose of 4 mg./kg. A similar fall in blood pressure was obtained by isochaksine given in a dose of 0.5 mg./kg. In some experiments the two drugs were combined in half these doses and injected intravenously in dogs. The results obtained were compared with the hypotensive responses obtained by injecting the two drugs separately.

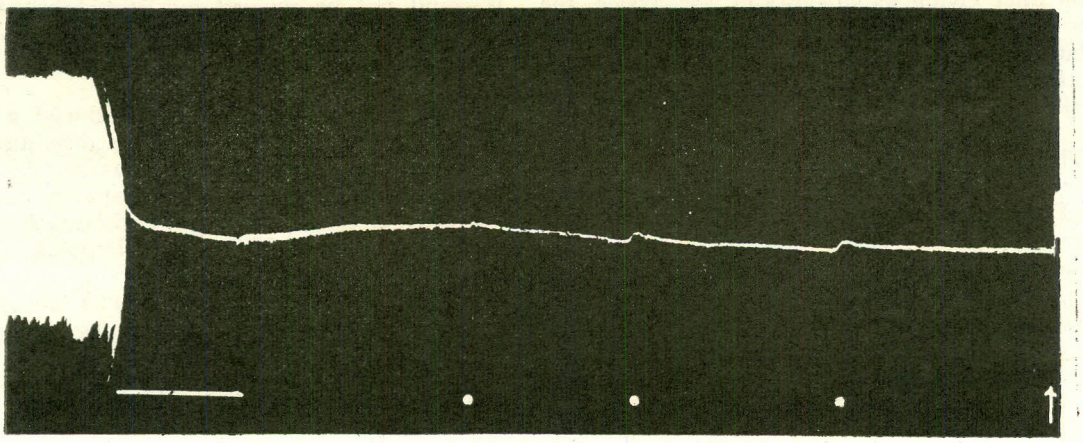

Fig. 1.—Isolated rabbit heart. Electrical stimulation made the heart fibrillate. Isochaksine 300 μg was injected at ● without any effect. Serpajmaline 200 μg was injected at ↑, and normal rhythm was obtained.

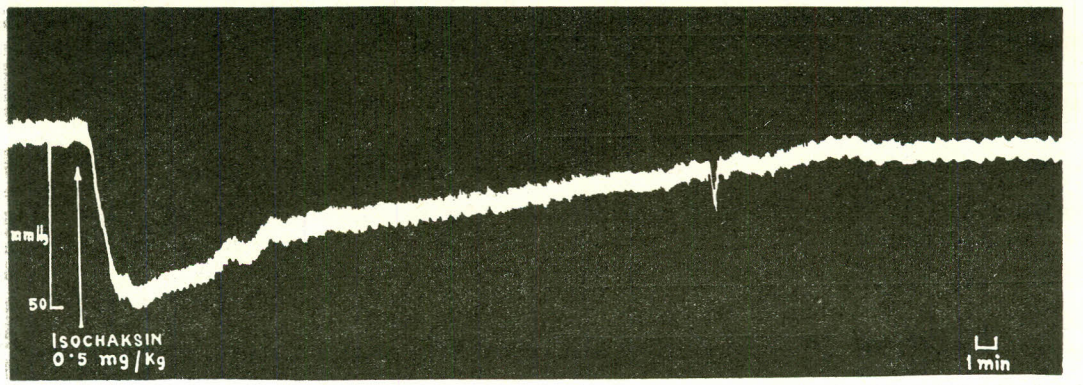

Fig. 2.—Dog blood pressure. Effect of intravenous injection of isochaksine 0.5 mg./kg.

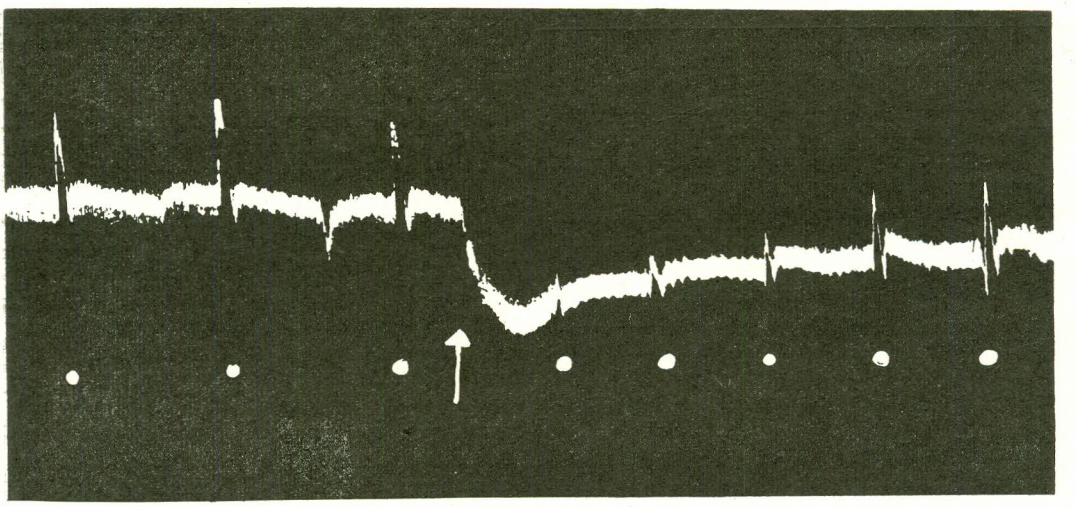

Fig. 3.—Dog blood pressure. Bilateral carotid occlusion for 30 seconds at ●. Isochaksine 0.25 mg./kg. injected intravenously at. ↑

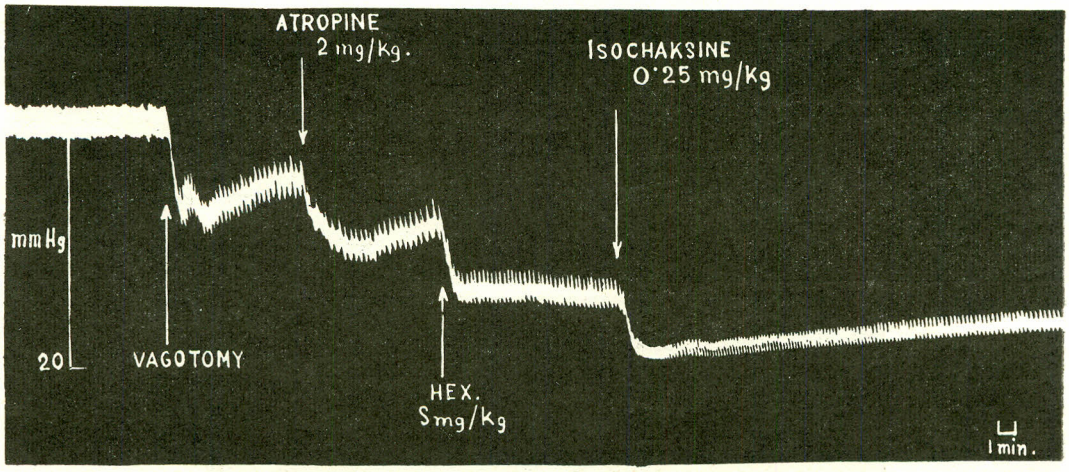

Fig. 4.—Dog blood pressure. Hypotensive action of isochaksine 0.25 mg./kg. after vagotomy, injection of atropine 2 mg./kg. and injection of hexamethonium 5 mg./kg.

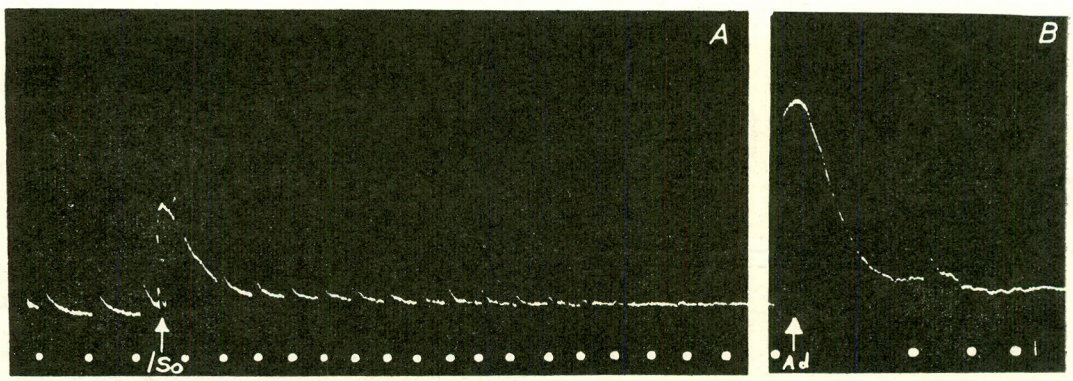

Fig. 5.—Cat nictitating membrane preparation. Electrical stimulation of the preganglionic sympathetic chain at ●. Isochaksine 0.25 mg./kg. injected at ↑ (A). Adrenaline Ad 20 μg remained effective after injection of isochaksine (B).

The combination of the two drugs produced a marked fall in the blood pressure of the dog. The blood pressure returned to control level slowly after about 30 minutes. Serpajmaline 4.0 mg./kg. and isochaksine 0.5 mg./kg. when given alone produced far less conspicuous results (Fig. 6).

Similar results were obtained in conscious dogs. The action of isochaksine 0.5 mg./kg. and serpajmaline 4.0 mg./kg. were studied on successive days. Isochaksine produced a longer lasting fall than serpajmaline. On the third day, a combination of the two drugs in half the stated doses was given intravenously. The resultant fall was greater than that seen after the two drugs given individually. This fall was also longer lasting. The results thus show that a kind of synergism occurs between the two drugs when given to conscious animals.

Discussion

Isochaksine produces a fall in the blood pressure of animals by a complex action. The fact that it brings about a marked reduction in the carotid occlusion response shows that the hypotensive action is partly central in origin. Isochaksine was also found to have hypotensive action after procedures like vagotomy, injection of atropine, and hexamethonium. These procedures blocked the reflex, lowering of the blood pressure, the post ganglionic cholinergic effects as well as the general automatic ganglia, repectively. It could therefore be said that the hypotensive response is partly due to a direct action on the vascular smooth muscle. The drug also blocks the impulses in the autonomic ganglia. This ganglion blocking action could certainly contribute to the hypotensive action. The direct inhibitory action on the cardiac musculature may also play a part.

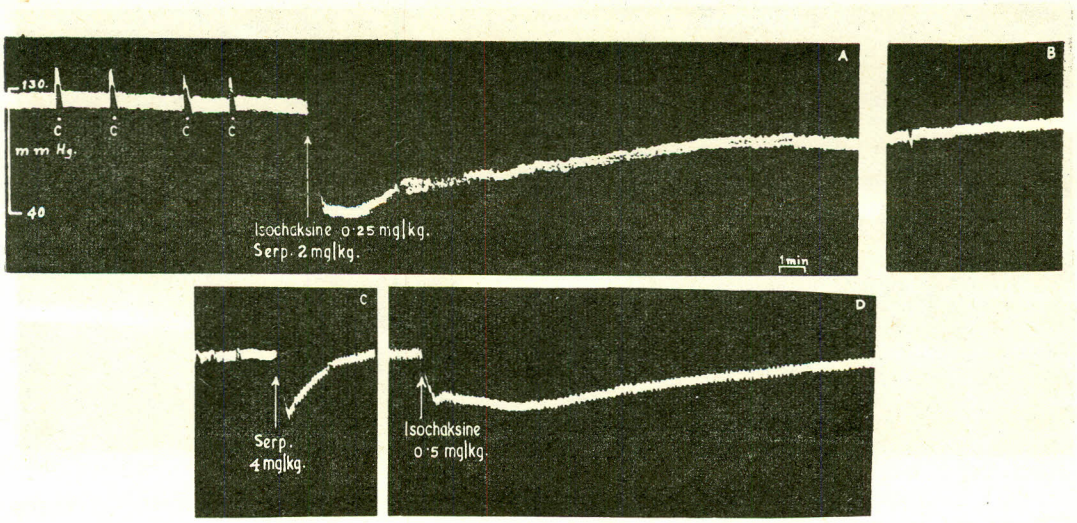

Fig. 6.—Dog blood pressure. Effect of intravenous injection of isochaksine and serpajmaline given together and separately.

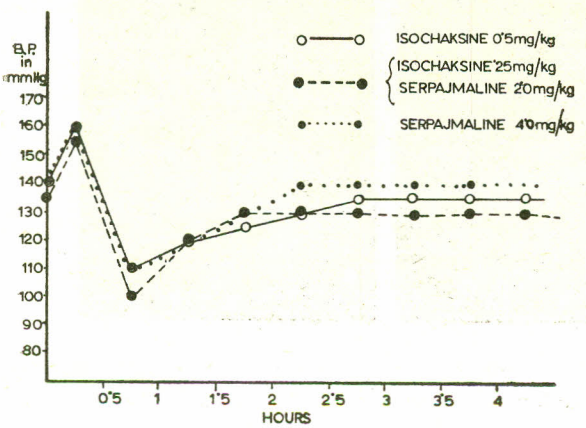

Fig. 7.—Systolic blood pressure of conscious dog. Effect of intravenous injections of isochaksine and serpajmaline. For details see text.

To sum up the cardiovascular actions of isochaksine it appears that it has a direct inhibitory action on the cardiac muscle. This is accompanied by constriction of the coronary arteries. It has no antifibrillatory action. It reduces the blood pressure by a complex action, which include both central as well as peripheral components. It blocks the preganglionic impulses in the autonomic ganglia. Isochaksine shows a synergistic action with serpajmaline on the blood pressure of anaesthetised as well as conscious animals.

The presence of another drug having more or less the same effects in the body often enhances the pharmacological actions of an active compound to an unexpected extent. This may be partially explained on the basis that there is some difference in the mechanism of action of the two drugs, thus making the mixture more efficient than any one of its components. Both serpajmaline and isochaksine have a central and a peripheral component in their hypotensive effect. Isochaksine, however, is also a ganglion blocking agent. It appears therefore probable that this ganglion blocking activity when combined with the central and peripheral hypotensive effects, produces a better pharmacological response than that of either of the two drugs given separately.

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