EFFECTS OF CHAKSINE CHLORIDE ON CHOLINERGIC AND TRYPTAMINE RECEPTORS IN THE ISOLATED GUINEA—PIG ILEUM

A. Q. S. Bukhari* and Inayat Khan†

North Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Peshawar

(Received March 13, 1963)

The stimulant and inhibitory actions of chaksine have been investigated on the isolated ileum of the guinea pig. The dose ratios to the various stimulant drugs (nicotine, 5-hydroxytryptamine, acetylcholine and histamine) were determined by keeping various concentrations of the antagonists in the bath for one hour and plotting the dose-response curves to the stimulant drugs before and after.

Chaksine chloride produced a weak antiacetylcholine effect. Chaksine showed a marked antinicotinic effect which increased with increase in the concentration of chaksine. It is a ganglion-blocking agent and also blocks nicotine receptors on the muscle cell. It is a potent anti 5-hydroxytryptamine agent and its increasing activity with the increase in the concentration indicates its dual action both at the muscle (D) receptors as well as at the nervous (M) receptors.

Isochaksine in a few experiments showed a much weaker atropine—like activity, antinicotinic, and anti-5-hydroxytryptamine activity. Further study with chaksine on the changes in behaviour of animals and human beings are suggested.

Introduction

Chaksine and isochaksine are two alkaloids obtained from the seeds of *Cassia absus Linn.*, called "Chaksu" in the vernacular. These seeds are used in indigenous medicine for treating various ailments, especially inflamatory conditions of the eye, and as an antispasmodic.

Mazharul Haq[1] showed that chaksine antagonized the stimulant effect of acetylcholine on isolated rabbit intestine. He attributed this effect to an atropine-like action.

Cheema[2] investigated the actions of isochaksine on the isolated intestine of rabbits and guinea-pigs; it did not antagonize barium chloride or histamine, whereas it antagonized acetylcholine slightly; but DMPP (a ganglion stimulating agent) markedly. It was therefore decided to investigate the actions of chaksine on isolated guinea-pig ileum in further details.**

Receptors

(*a*) *Acetylcholine-sensitive Receptors.*—These receptors exist at two distinct places in the guinea-pig-ileum: (i) *In the autonomic ganglia* where they are the same as the nicotine receptors. Both acetylcholine and nicotine, in suitable doses stimulate these receptors leading to stimulation of the smooth muscle. These receptors are blocked by ganglion blocking agents, like hexamethonium and also by large doses of nicotine but not by atropine. (ii) *In the smooth muscles* there is another set of acetylcholine-sensitive receptors, referred to as "muscarine" receptors, which are blocked by atropine-like drugs.

There is also another type of nicotine-sensitive receptors, quite distinct from the muscarine type. They are not affected by atropine or hexamethonium but are blocked by 5-benzyloxy-NN-dimethyl tryptamine.[3]

(*b*) *Tryptamine Receptors.*—5-Hydroxytryptamine (sertotonin) is a pharmacologically active amine present in various parts of the human body e. g., in the grey matter of the brain and in blood platelets.[4] It seems to play an important role in the body. It is believed that patients suffering from various psychological disorders, or from carcinoid tumours, have a deranged 5-hydroxytryptamine metabolism. Persons bitten by scorpions or by wasps suffer from effects of large doses of 5-hydroxytryptamine (besides other active agents) because the venoms of scorpions and wasps contain large quantities of 5-hydroxytryptamine, whereas bee-venom is devoid of it. Analogues of 5-hydroxytryptamine, especially its antagonists may prove useful in conditions due to derangement of 5-hydroxytryptamine metabolism and thus pharmacologists are on the look out for simple tissues for screening these compounds.

The isolated guinea-pig ileum, has been used for investigating the actions of analogues of 5-hydroxytryptamine. 5-Hydroxytryptamine-sensitive receptors in this preparation are called tryptamine receptors.[4] Tryptamine, an analogue of 5-hydroxytryptamine, is another naturally occurring amine with actions somewhat similar to those of 5-hydroxytryptamine.

* Now at Pharmacology Section, Central Laboratories, P.C.S.I.R., Karachi.

† Now at Department of Pharmacology, Jinnah Postgraduate Medical Centre, Karachi.

** A paper entitled "Pharmacological actions of Chaksine and isochaksine on tissues other than guinea-pig ileum" was read at the XIV All Pakistan Science Conference at Peshawar.

Gaddum and Picarelli [5] showed that there are two types of tryptamine receptors in the guinea-pig ileum. One, in the smooth muscle, sensitive to dibenzyline and hence is called the "D" receptor, while the other type, in nervous tissue, sensitive to morphine and therefore is called the "M" receptor. The location of the M receptors seems to be more peripheral than the ganglion cells, probably somewhere on the post-ganglionic fibres, and as a result of stimulation of these receptors by 5-hydroxytryptamine, acetylcholine is released at the post-ganglionic nerve endings; an effect similar to th stimulation of nicotine receptors at the ganglion, except that the latter is blocked by cocaine or by morphine. Morphine prevents the release of acetylcholine from post-ganglilnic nerve endings[6] and thus blocks the effects of both 5-hydroxytryptamine and nicotine on the nervous tissue receptors.

Methods

Drugs Used.—(i) 5-Hydroxytryptamine creatinine sulphate supplied as a gift by M. E. Speeter of the Upjohn Company, U. S. A. and M/S. E. Merek, A. G.—Darmstadt. (ii) Chaksine chloride and isochaksine were supplied by the Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi. (iii) Histamine acid phosphate, hexamethonium bromide, acetylcholine chloride, nicotine hydrogen tartrate and morphine sulphate. (iv) All the drugs were calculated as salts, except 5-hydroxytryptamine creatinine sulphate and morphine sulphate, which were calculated as bases.

Experimental

Terminal segments of ileum from guinea-pigs were suspended in a 10 ml. bath, containing well oxygenated Tyrode's Solution, at 37°C. The various stimulant drugs were used to obtain their respective dose responses, then a fixed concentration of an antagonist was maintained in the bath for one hour, and during this period the doses of the stimulant drugs were repeated; and their doses were increased if their responses were depressed by the antagonist. The degree of depression was estimated by comparing the responses of antagonists, before and after applying the antagonist, especially at the end of one hour. The ratio between the two doses of the antagonist producing the same responses, is termed the Dose-Ratio,[7] as shown in Figure 1. It is directly proportional to the degree of antagonism.

Results

Antagonistic Effect of Hexamethonium and Morphine.—Hexamethonium: A concentration of 10 μg./ml. of this drug produced maximum antagonism to the actions of nicotine only ; and on increasing the concentration of hexamethonium to 100 μg./ml. no significant increase in its antagonism was noted against any of the drugs as shown in Figure 2 (i and ii). This is because it is only the ganglionic effects of nicotine which are blocked by hexamethonium whereas the effect of nicotine on the smooth muscle is resistant to hexamethonium and so there is no further increase in its dose ratio shown in Table 1.

Morphine: Table 2 shows clearly that morphine in a concentration of 1 μg./ml. antagonized the effects of nicotine and 5-hydroxytryptamine only. By increasing the concentration of morphine 10 times, i. e. to 10 μg./ml.there is no further increase in the antagonism to any of the drugs because both nicotine and 5-hydroxytryptamine now act on the receptors located in the smooth muscles which are not sensitive to morphine.

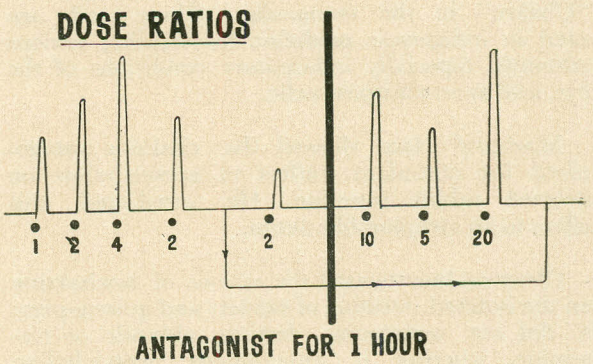

Fig. 1.—Responses to a stimulant drug before and after an antagonist, which was maintained in the bath for one hour. The doses of the stimulant drug which were proportional to the units, indicated, had to be increased in the presence of the antagonist, to get the same responses. For example 20 units after the antagonist produced a response equal to 4 units before. The dose ratio in this experiment is 20/4=5.

Actions of Chaksine

Antagonistic Effects.—Chaksine in the concentrations of 10 ug./ml. to 100 μg./ml. does not show any stimulant activity on this preparation.

Antagonistic Effects of Chaksine.—Chaksine in a concentration of 1 μg./ml., the minimal effective concentration, produced marked depression of responses to nicotine, acetylcholine and 5-hydroxytryptamine but not to histamine. It is clear from Table 3 that on increasing the concentration of chaksine to 10 or 100 μg./ml. its antagonism of

nicotine, acetylcholine and 5-hydroxytryptamine increased; while histamine still remained unaffected Figure 3 (i and ii).

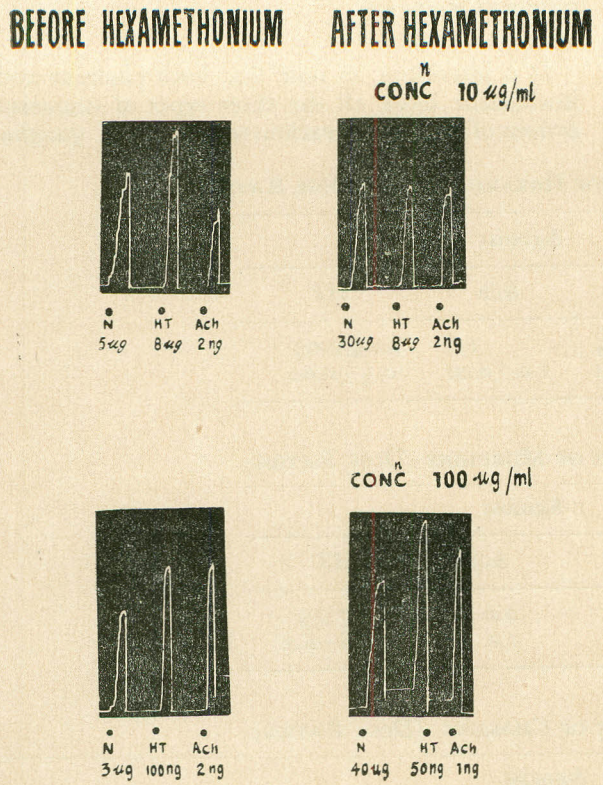

Fig. 2.—Isolated Guinea pig ileum:—(i) Responses to nicotine (N) 5-hydroxytryptamine (HT) and acetylcholine (Ach) before and after 10 ug./ml. of hexa-methonium. In this experiment there is 6 times depression of nicotine and 1.5 times of 5-hydroxytryptamine but acetylcho-line responses are unaffected.

(ii) The above responses before and after 100 μg./ml. of hexamethonium. There is 10 times depression of nicotine while 5-hydroxytryptamine and acetylcholine responses are slightly increased.

Effects of Chaksine on Acetylcholine-sensitive Receptors.- (a) Antimuscarinic Effect : Chaksine in all the three concentrations used, showed a definite and increasing degree of anti-acetylcholine activity. More than one thousand molecules of chaksine chloride produced an effect equal to that of one molecule of atropine sulphate. (b) Antinicotinic Effects: Chaksine had a marked antinicotinic effect starting at a concentration of 1 μg./ml. and the degree of antagonsim increased significantly with the increase in the concentration of chaksine, which is a clear evidence that chaksine is not only a ganglion-blocking agent but also blocks the second type of nicotine receptors at the muscle cell level. Thus its antinicotinic effect is different from that of hexamethonium.

Effects on Tryptamine Receptors.—Chaksine 1 μg./ml. blocked the responses to 5-hydroxytryptamine. By increasing the concentration of the antagonist to 10 or 100 ug./ml., its specific anti-5-hydroxytryptamine effect was also increased.

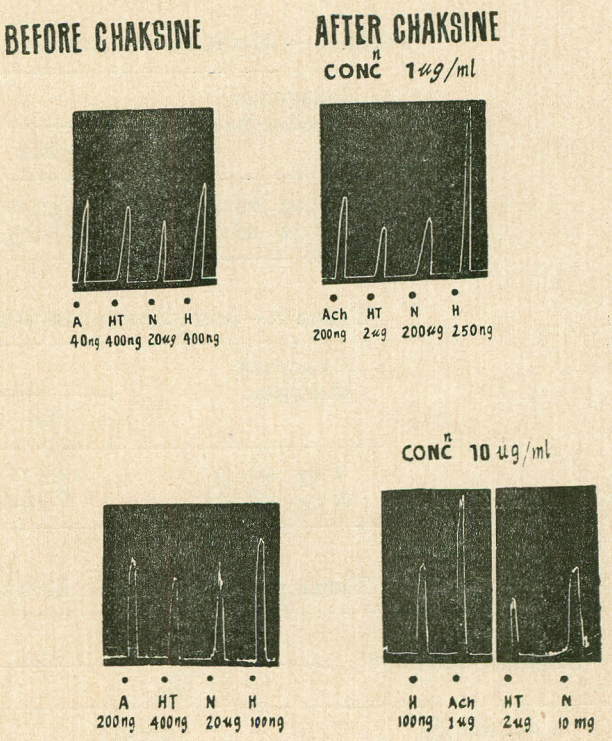

Fig. 3.—Isolated guinea-pig ileum:—(i) Responses to acetylcholine (A) 5-hydroxytryptamine (HT) nicotine (N), and histamine (H) are shown before and after 1 ug./ml. of chaksine. There is 5 times depression of acetylcholine, about 5 times of 5-hydroxytryptamine and 10 timesof nicotine. Histamine responses are slightly increased.

(ii) The concentration of chaksine used is 10 μg./ml. There is about 5 times depression of acetylcholine, about 10 times of 5-hydroxytryptamine, 500 times of nicotine, and about 2 times of histamine.

Thus it acts on both the muscle (D) receptors and nervous (M) receptors. If it acted on any one of the two receptors, an increase in the concentration of chaksine after a certain level, would not have produced further increase in antagonsim to 5-hydroxytryptamine, as with morphine sulphate.

ACTIONS OF ISOCHAKSINE

The results of a few similar dose-ratio experiments with isochaksine are shown in Table 4.

Below 10 μg./ml. marked antinicotinic action was accompanied by some atropine like activity and also by anti-5-hydroxytryptamine effects. Histamine responses were not significantly reduced. The weak atropine-like activity of isochaksine and of chaksine are in agreement with the results of Cheema[2] and of Hye and Wahid.[8] Chaksine is about ten times more potent than isochaksine in blocking responses to nicotine on this tissue. This antinicotinic activity is partly an indication of the drug's ganglion-blocking action; hexamethonium also shows a weak antinicotinic effect on this preparation.

Discussion

The weak atropine-like activity of chaksine and isochaksine does not explain or justify the use of "Chaksu" as an antispasmodic in the indigenous system of medicine.

It is interesting to note that both chaksine and isochaksine block all the three types of stimulant actions of nicotine at various sites : (a) at ganglia both drugs are ganglion blocking agents. (b) at neuromuscular junctions both are muscle relaxants.[9] (c) smooth muscle fibres in the

TABLE 1.— ANTAGONISTIC ACTIVITY OF HEXAMETHONIUM (DOSE RATIOS)

Antagonist Hexamethonium	Agonist		
	Nic	Ach	HT
10 μg. ml.(3)	6.3±0.5	1.0	1.2±0.03
100 μg. ml.(3)	10.6±5.1	1.0±0.02	0.5±0.02

TABLE 2.—ANTAGONISTIC EFFECTS OF MORPHINE (DOSE RATIOS).

Antagonist Morphine	Agonist		
	Nic	Ach	HT
1 μg. ml. (3)	13±11	1.0	7±3
10 μg. ml. (3)	8±2.2	1.0	16±2.2

TABLE 3.—ANTAGONISTIC EFFECT OF CHAKSINE (DOSE RATIOS).

Antagonist Chaksine	Agonist			
	Nic	Ach	HT	Hist
1 μg. ml. (6)	13±13.5	5±1.1	5.5±1.5	1.5±0.4
10 μg. ml.(5)	970±47	17±14	31.0±6	2.2±0.1
100 μg. ml. (4)	>1,000 > 10,000. >10,000> 10,000.	36.2±16	134±17	3.1±1

TABLE 4.—ANTAGONSTIC EFFECTS OF ISOCHAKSINE (DOSE RATIOS).

Antagonist Isochaksine	Agonist			
	Nic	Ach	HT	Hist
1 μg. ml.	0.75	0.75	0.5	0.5
10 μg. ml.(3)	10±5	2.7±1.55	2.9±1.45	1.25±.2
100 μg. ml.(3)	2333±88.6	12.6±3.8	16.4±1.6	2.75±0.8

Note.—Numerals in the brackets show the numbers of experiments performed. The average dose ratios to Nicotin e (Ni.c) Acetylcholine (Ach) 5-Hydroxytryptamine (HT) and Histamine (Hist) are shown with standard errors. This also applies to Table 1,2 3, and 4.

guinea-pig ileum; the significance of this is not yet fully understood.

The hypotensive effects of chaksine and isochaksine, in anesthetized animals, do not differ significantly[9] but on the guinea-pig ileum the higher dose ratios of chaksine to nicotine and 5-hydroxytryptamine may be due to an increased activity of chaksine on both the nicotine and 5-hydroxytryptamine receptors of the muscle fibre.

The anti-5-hydroxytryptamine activity of chaksine seems to be on both types of tryptamine receptors including the nervous tissue (M) receptor. In a few experiments on the rat uterus preparation, chaksine blocked 5-hydroxytryptamine contractions.

Cheema's[2] finding that isochaksine potentiated the sleep-producting effects of pentobarbitone and hexobarbitone in rats, is an interesting clue to the central effects of these compounds. Chaksine, a more potent antagonist of 5-hydroxytryptamine on M receptors, i. e. the nervous tissue receptors, requ res further study on animals and human beings. It may produce interesting changes in behaviour.

Acknowledgements.—Thanks are due to Dr. S. A. Warsi, Director, North Regional Laboratories, Peshawar, for his keen interest and encouragement.

References

1. Mazharul Haq, Medicus, **6,** 215 (1952).
2. M. A. Cheema, *Proc. XIV. Pak. Sci. Conf.* (1962)
3. R. B. Barlow, and I. Khan, *Brit. J. Pharmacol*, **14,** 533 (1959).
4. J. H. Gaddum, *J. Physiol. (London)* 119, 363, (1953).
5. J. H. Gaddum Z. P. Picarcelli, *Brit. J. Pharmacol*, **12,** 323 (1956).
6. W. D. M. Paton, *J. Physiol, (London)*, **137,** 35 (1057).
7. J. H. Gaddum, K.A. Hameed, D. E. Hathsway and F. F. Stephens, *Quart. J. Exp. Physiol*, **40,** 49 (1955).
8. H.K.M. Hye and M.A. Wahid, Pakistan J. Sci. Ind. Research, **5,** 6, (1962).
9. I. Khan, A. Q. S. Bukhari, and Mujahid Hussain, *Proc. XIV Pak. Sci. Conf.* (1962).