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STIMULATORY EFFECT OF TOLAZOLINE ON SMOOTH MUSCLE

BY

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It is known that tolazoline not only blocks the effect of sympathomimetic drugs, but may also increase responses to sympathetic nerve stimulation (Varagić, 1956 ; Boyd, Chang & Rand, 1960 ; Burn & Gibbons, 1964 ; Burn & Ng, 1965). Benfey & Varma (1964) showed that tolazoline itself causes a rise in blood pressure in reserpinized cats which may be a direct sympathomimetic effect.

It has also been reported that tolazoline produces parasympathomimetic effects probably due to its anticholinesterase activity (Gowdey, 1948 ; Mizuno, 1959 ; Boyd *et al.*, 1960 ; Sharma, Dasputra, Jayaswal & Grewal, 1964 ; Burn & Gibbons, 1964). However, on some cholinergically innervated preparations such as the guinea-pig rectum, or toad bladder, tolazoline blocks the response to both stimulation of pelvic nerve and to added acetylcholine (Boyd, Burnstock, Cambell, Jowett, O'Shea & Wood, 1963).

These conflicting results on cholinergically innervated preparations prompted the present investigation into the effect of tolazoline on the transmurally stimulated guinea-pig ileum (Paton, 1957 ; Harry, 1962).

METHODS

Transmurally stimulated guinea-pig ileum

The technique of Paton (1957) was followed. The bath capacity was 20 ml. Segments of gut were suspended in Krebs solution bubbled with oxygen. Single pulses of 1 msec duration and 20 V at a rate of 6 shocks/min were used to stimulate the gut. The contractions of the ileum were recorded on a smoked drum.

Longitudinal movements of isolated guinea-pig ileum

Segments 5–6 cm were taken from the ileum 10 cm from the ileocaecal junction and were suspended in a 20 ml. organ bath. Krebs solution at 37° C was gassed with oxygen. When the muscle was depolarized by immersion in calcium-free K_2SO_4-Ringer, the solution had the following composition (mM): K_2SO_4 126.0, KCl 5.6, $KHCO_3$ 3.6, glucose 5.5.

Treatment with reserpine

Guinea-pigs were injected intraperitoneally with reserpine (2 mg/kg) 48 hr and 24 hr before the experiments.

Drugs

The following drugs were used: acetylcholine hydrochloride, adrenaline hydrochloride, angiotensin (Hypertensin, Ciba), antazoline hydrochloride, atropine sulphate, barium chloride, bretylium tosylate, cocaine hydrochloride, dihydroergotamine methanesulphonate, guanethidine sulphate, hemicholinium, hexamethonium bitartrate, histamine phosphate, 5-hydroxytryptamine creatinine sulphate, isoprenaline hydrochloride, morphine hydrochloride, noradrenaline hydrochloride, papaverine hydrochloride, phentolamine hydrochloride, potassium chloride, procaine hydrochloride, promethazine hydrochloride, pronethalol hydrochloride, reserpine (Serpasil, Ciba), tolazoline hydrochloride (Priscol, Ciba), yohimbine hydrochloride.

Doses of drugs are expressed in terms of salts.

RESULTS

Transmurally stimulated guinea-pig ileum

Tolazoline. The addition of tolazoline (2–20 μg/ml.) to the bath induced an increase of responses of ileum to electrical stimulation (Fig. 1). However, the tolazoline used in concentration up to 5 μg/ml. regularly augmented the tonus of the ileum (Fig. 1b). When higher doses of tolazoline (20–200 μg/ml.) were added to the bath, the rise of the tonus was followed by occasional contractions. The potentiating effect of tolazoline developed rapidly and was proportional to the dose of tolazoline used (2–20 μg/ml.). The effect

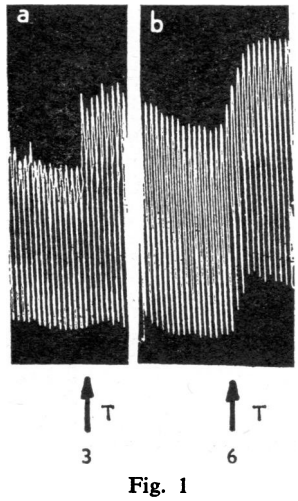

Fig. 1

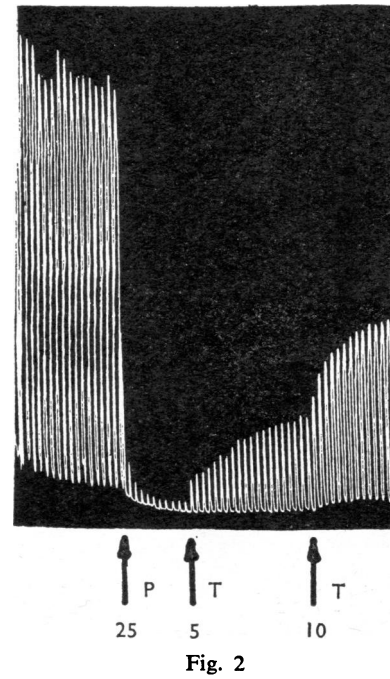

Fig. 2

Fig. 1. Transmurally stimulated guinea-pig ileum. At ↑ T tolazoline was added to the bath. In this and subsequent tracings the number below the arrow refers to the concentration of substance in the bath (μg/ml.).

Fig. 2. Transmurally stimulated guinea-pig ileum. The blockade of responses to transmural stimulation by procaine (↑ P). At ↑ T, tolazoline was added to the bath.

was reversible. After washing tolazoline out of the bath the potentiation disappeared in 5 to 10 min.

Local anaesthetics. Cocaine (50 μg/ml.) and procaine (2–30 μg/ml.) had an inhibitory effect on the responses of the transmurally stimulated guinea-pig ileum. This inhibitory effect of cocaine and procaine has been reported by Paton (1955) and Harry (1962). In our experiments the inhibitory effect of cocaine and procaine was antagonized by tolazoline (Fig. 2). However, after 20–30 min of continuous contact of guinea-pig ileum with procaine, the tolazoline failed to increase the responses of ileum to electrical stimulation leaving the action of histamine (0.05 μg/ml.) unaffected.

Sympathomimetic amines. Noradrenaline (0.1–5 μg/ml.), adrenaline (0.1–2 μg/ml.), and isoprenaline (1–2.5 μg/ml.) inhibited the contractions of guinea-pig ileum. Tolazoline (2–5 μg/ml.) antagonized the inhibitory effect of these substances (Fig. 3).

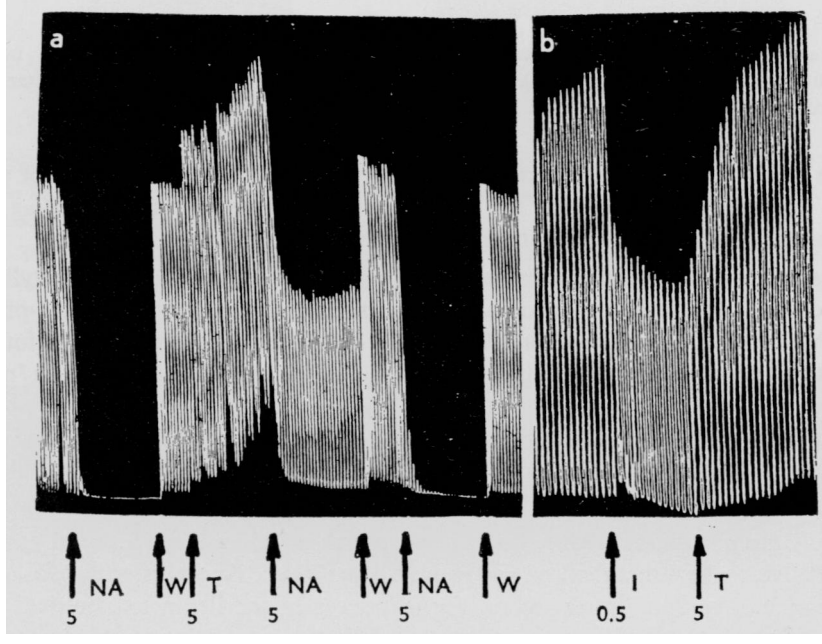

Fig. 3. Transmurally stimulated guinea-pig ileum. The blockade of responses to transmural stimulation by noradrenaline (↑ NA) and isoprenaline (↑ I). At ↑ T, tolazoline was added to the bath. At ↑ W indicates wash.

Substances blocking adrenergic alpha- and beta-receptors. Khairallah & Page (1962) showed that phentolamine, piperoxane and dichloroisoproterenol decreased the responses to electrical stimulation of guinea-pig ileum. We found in addition that a similar effect could be exerted by yohimbine (2 μg/ml.), dihydroergotamine (0.5 μg/ml.) and pronethalol (2 μg/ml.). The inhibitory effects of these three substances and phentolamine (2–10 μg/ml.) were antagonized by tolazoline in doses from 2 to 10 μg/ml. (Figs. 4a, 4b). Addition of phentolamine (10 μg/ml.) and pronethalol (2 μg/ml.) to the bath together, did not abolish the stimulatory effect of tolazoline (10–20 μg/ml.) on the ileum

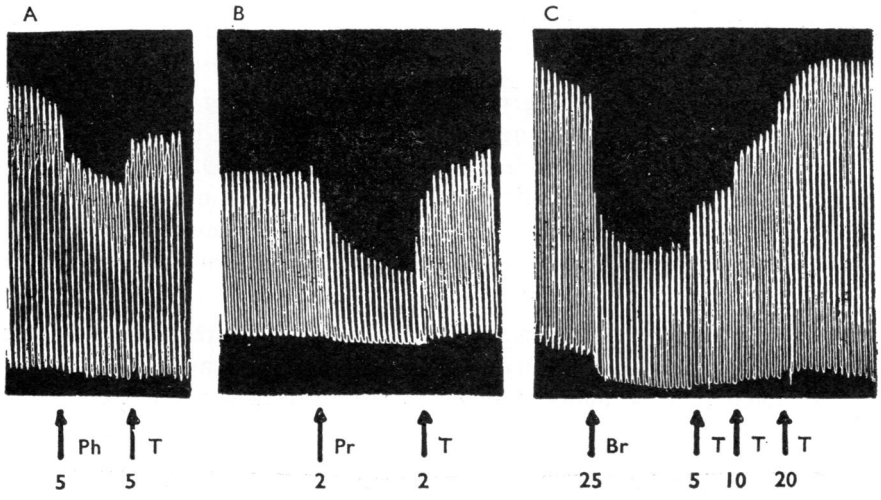

Fig. 4. Transmurally stimulated guinea-pig ileum. The blockade of responses to transmural stimulation by phentolamine (↑ Ph), pronethalol (↑ Pr) and bretylium (↑ Br). After addition of tolazoline (↑ T) to the bath the responses were enhanced.

taken from either reserpinized or normal animals. The same concentrations of phentolamine and pronethalol antagonized the inhibitory effect of noradrenaline (0.1 μg/ml.) or isoprenaline (0.1 μg/ml.) on the transmurally stimulated guinea-pig ileum.

Adrenergic neurone blocking substances and reserpine. Guanethidine, bretylium and reserpine caused progressive blockade of transmural stimulation of guinea-pig ileum (Khairallah & Page, 1962 ; Dandiya, 1963). We confirmed these results and found that the inhibitory effects of guanethidine (10–20 μg/ml.), bretylium (10–25 μg/ml.) and reserpine (1–5 μg/ml.) could be antagonized by tolazoline (5–20 μg/ml.). A typical experiment with bretylium is shown in Fig. 4c. However, in reserpine treated animals the responses of the ileum to electrical stimulation were not significantly changed and the stimulatory effect of tolazoline still persisted.

Hexamethonium. Paton (1955) showed that twitches induced by electrical stimulation were insensitive to hexamethonium. However, Härtfelder, Kuschinsky & Mosler (1958) reported that hexamethonium depresses the responses of the ileum to electrical stimulation. We confirmed the results of Paton and found that the potentiating effect of tolazoline (2–20 μg/ml.) was not influenced by prior addition of hexamethonium (2–150 μg/ml.) to the bath (Fig. 5).

Atropine. The twitches could be reduced by atropine (Paton, 1955, 1956, 1957). However, the inhibited responses of guinea-pig ileum to transmural stimulation by atropine (0.01–0.1 μg/ml.) could again be partly reversed after addition of tolazoline in concentrations of 5–20 μg/ml. (Fig. 6). Higher doses of atropine (0.1–5 μg/ml.) reduced or abolished the stimulatory effect of tolazoline (2–20 μg/ml.).

Hemicholinium. When hemicholinium (50 μg/ml.) was added to the bath the responses to electrical stimulation during a 30 min period were reduced. At the end of this period, addition of tolazoline (5–20 μg/ml.) failed to reverse the inhibitory effect of hemicholinium.

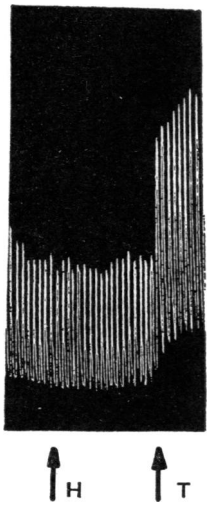

Fig. 5

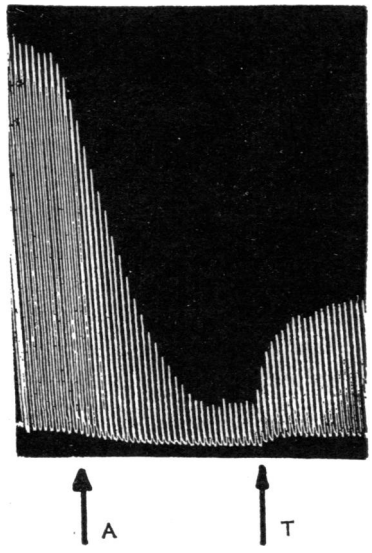

Fig. 6

Fig. 5. Transmurally stimulated guinea-pig ileum. At ↑ H hexamethonium (75 μg/ml.) was added to the bath. Tolazoline (↑ T) in a concentration of 5 μg/ml. enhanced the contractions induced by electrical stimulation of guinea-pig ileum.

Fig. 6. Transmurally stimulated guinea-pig ileum. The blockade of responses to transmural stimulation by atropine ↑ A (0.01 μg/ml.). At ↑ T tolazoline (5 μg/ml.) antagonized the inhibitory effect of atropine.

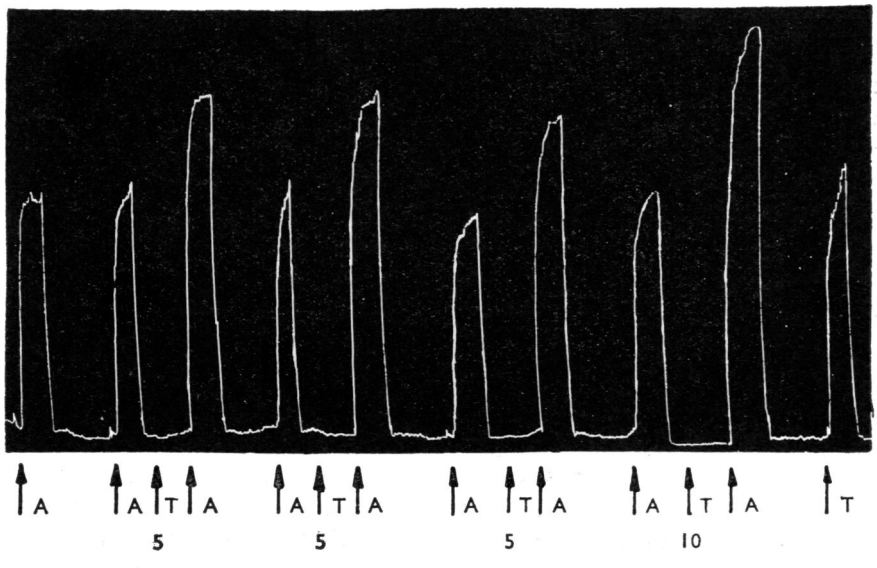

Fig. 7. Isolated guinea-pig ileum. Contractions in response to addition of angiotensin (↑ A)—bath concentration 0.1 ng/ml. They were potentiated by addition of tolazoline (↑ T) 2 min before angiotensin. Contact time of angiotensin with ileum 2 min.

Antihistaminics. Antihistaminic drugs, antazoline (0.1–0.3 μg/ml.), and promethazine (0.1–0.5 μg/ml.) had no effect either on the contractions of guinea-pig ileum induced by electrical stimulation or on the stimulatory effect of tolazoline (5 μg/ml.). These doses of antihistaminics reduced the motor effect of histamine (0.05 μg/ml.) on the longitudinal muscle of guinea-pig ileum.

Longitudinal movements of guinea-pig ileum

Tolazoline and various smooth muscle stimulants. In most of our experiments tolazoline in a concentration of 2 μg/ml. did not induce any change of muscle tonus of guinea-pig ileum. Higher doses of tolazoline up to 5 μg/ml. induced contraction. This effect could be abolished by atropine (0.1–5 μg/ml.). Very large concentrations of tolazoline (800 μg/ml.) have been found to block completely the response to acetylcholine (Sharma *et al.*, 1964). We confirmed this observation, but found that lower concentrations (2.5–50 μg/ml.) potentiated the responses to acetylcholine (0.5–2 μg/ml.). A similar potentiating effect of tolazoline (5–40 μg/ml.) could be observed also on the contractions induced by angiotensin (0.1 ng/ml.) (Fig. 7), barium chloride (100–250 μg/ml.), potassium chloride (200–500 μg/ml.) and 5-hydroxytryptamine (0.1 μg/ml.). This effect was antagonized by atropine (0.1–5 μg/ml.). However, the blockade of receptors in nervous tissue by previous addition of morphine (Gaddum & Picarelli, 1957) in doses of 1–10 μg/ml. did not abolish the stimulatory effect of tolazoline (10–40 μg/ml.) on the 5-hydroxytryptamine (1–2 μg/ml.) induced contraction.

The results concerning the influence of tolazoline on histamine (0.01–0.05 μg/ml.) induced contractions were variable. In some experiments it was found that tolazoline in doses 2–20 μg/ml. exerted a potentiating effect, while in others, the responses of the ileum to histamine were either reduced or not changed by prior addition of tolazoline. However, higher doses of tolazoline (20–160 μg/ml.) always antagonized the stimulatory effect of histamine. This antagonism was reversible. The analysis of cumulative dose-response curves of histamine showed that in the presence of a range of concentrations (20–160 μg/ml.) of antagonist (tolazoline), there is parallel shift to the right of dose-effect curves of agonist (histamine). In these experiments it was evident that the maximum stimulatory effect of histamine in the presence of tolazoline was not reduced if the dose of histamine was sufficiently increased.

Depolarized preparation. Contractions induced by acetylcholine (0.5–50 μg/ml.) on depolarized guinea-pig ileum could not be increased or reduced by tolazoline (5–100 μg/ml.). Tolazoline in doses 5–100 μg/ml. failed to change the tonus of muscle.

DISCUSSION

In the present study it was shown that many substances which reduce the twitches induced by electrical stimulation were antagonized by tolazoline. We have demonstrated also that the stimulatory effects of tolazoline persist after treating the animal with reserpine or after the addition of alpha and beta receptor blocking agents to the bath. These results include the possibility that the stimulation is due to the anti-adrenaline properties of tolazoline. In addition, dibenamine had no effect on the responses of isolated intestinal strips to electrical stimulation. Other antagonists such as phentolamine or dichloroiso-

prenaline even decreased the ileum responses (Khairallah & Page, 1962). All these results indicate that anti-adrenaline properties of tolazoline are not essential for its stimulatory effect in electrically stimulated guinea-pig ileum.

The stimulant effect of the tolazoline on the ileum could be also ascribed to its effect on ganglion cells, but this hypothesis is unlikely as the stimulating effect of tolazoline still persists in the presence of hexamethonium.

Some effects of tolazoline have been attributed to its histamine-like action (Nickerson, 1949) so the antihistaminic drugs, antazoline and promethazine, were used to analyse the stimulatory effect of tolazoline. It was shown that antihistaminic drugs failed to reduce the contracting effect of tolazoline.

It thus seems reasonable to suppose that the stimulatory effect of tolazoline is due either to a rise of concentration of transmitter substances or direct sensitisation of smooth muscle cells to the acetylcholine released during electrical stimulation. This was suggested by the fact that tolazoline blocked the action of many substances with direct inhibitory effects on smooth muscle. Harry (1962) showed that papaverine abolished the contractions of electrical stimulation without reducing the output of acetylcholine. Atropine has a similar effect with specific blockade of acetylcholine receptors. The blocking effect of other drugs used in our experiments is not yet clear but much evidence indicates the direct inhibitory effect on smooth muscle. Thus, Kosterlitz & Lees (1961) reported that the blocking effect of bretylium on the peristaltic reflex of the guinea-pig ileum is due to weak atropine-like action. It was also shown by the same authors that bretylium inhibits the contractions of longitudinal muscle caused by histamine, carbachol, nicotine, acetylcholine and 5-hydroxytryptamine. Such a non-specific inhibitory effect was also reported for guanethidine (Garrett & Sousa, 1963; Džoljić, 1965). Dandiya (1963) suggested that the inhibitory effect of reserpine on the twitches of transmurally stimulated guinea-pig ileum is the result of depressant action on smooth muscle and competitive action on acetylcholine. However, Khairallah & Page (1962) suggested that inhibition of the twitches could be caused by the liberation of catecholamines from intracellular stores by reserpine, guanethidine and bretylium.

Some of the adrenaline antagonists could inhibit the response of the intestinal strips by their sympathomimetic activity although other explanations, such as direct effect on the smooth muscle cells cannot be excluded (Khairallah & Page, 1962). It also could be suggested that yohimbine (Nickerson, 1949) and pronethalol (Gill & Vaughan Williams, 1964), as is assumed to be the case with bretylium (Boura & Green, 1959), blocks the post-ganglionic nerve terminals through a local anaesthetic effect.

It is evident that the mode of inhibitory action of all these drugs on transmurally stimulated guinea-pig ileum is not yet satisfactorily solved. However, the stimulatory effect of tolazoline on the blocking activity of such different drugs could be satisfactorily explained on the basis of accumulation of acetylcholine on the receptor sites as a result of tolazoline anticholinesterase activity. The evidence for the anticholinesterase activity of tolazoline on the extract of vas deferens and rabbit serum has been already published (Boyd *et al.*, 1960; Sharma *et al.*, 1964). Anticholinesterase activity of tolazoline demonstrated by biological assay methods was confirmed later by manometric experiments (Burn & Gibbons, 1964).

Contractions of the guinea-pig ileum induced by transmural stimulation were unaffected by tolazoline after procaine and hemicholinium had been in contact with the organ for 30 min. Harry (1962) showed that procaine reduced the acetylcholine contractions without reducing the sensitivity of the muscle to the test dose of histamine. It has been also shown by many authors (Reitzel & Long, 1959 ; Chang & Rand, 1960 ; Wong & Long, 1961) that hemicholinium reduces the release of acetylcholine from cholinergic nerve endings. The fact that tolazoline failed to stimulate the contractions of the ileum reduced by prior addition of procaine or hemicholinium suggests that the potentiating effect of tolazoline could be related to the concentration of the released cholinergic transmitter. A direct inhibitory effect of procaine on the ileum was excluded since the response to test doses of histamine in the presence of procaine was not reduced.

The potentiating effect of tolazoline on contractions induced by other stimulant drugs such as angiotensin, barium chloride, potassium chloride and 5-hydroxytryptamine, could also be ascribed to the anticholinesterase activity of tolazoline.

The direct sensitizing effect of tolazoline on smooth muscle cells to various stimulants should also be considered. Obviously, many sympathomimetic effects of tolazoline such as tachycardia, potentiation of the vasoconstrictor effect of barium chloride (Nickerson, 1949), etc., cannot be explained on the basis of anticholinesterase activity of tolazoline. However, the fact that complete depolarization of the muscle membrane by immersion in the high potassium solution abolished the stimulatory effect of tolazoline, indicates that a site of action of tolazoline is not inside the cell. Further evidence that the stimulatory effect of tolazoline on transmurally stimulated guinea-pig ileum and longitudinal muscle was blocked by atropine, indicate that stimulation of the muscle is mediated through the cholinergic receptors. These findings suggest that specific stimulation of the cholinergic system, probably indirectly due to anticholinesterase action, is essential for the stimulatory effect of tolazoline. It seems, therefore, that there is no justification for postulating a nonspecific direct sensitizing effect of tolazoline on the guinea-pig ileum.

Concerning the inhibitory action of tolazoline on the contractile effect of histamine it could be suggested that tolazoline, which is chemically similar to histamine, competes for the same receptors as histamine. Our results with cumulative dose-response curves of histamine in the presence of various concentrations of tolazoline support the view of competitive antagonism of tolazoline.

SUMMARY

1. The effect of tolazoline on the guinea-pig isolated ileum stimulated electrically or by drugs has been studied. Tolazoline potentiated the responses of transmurally stimulated guinea-pig ileum taken from normal or reserpinized animals. This effect was antagonized by atropine.

2. The inhibitory effect of cocaine, noradrenaline, adrenaline, isoprenaline, phentolamine, yohimbine, dihydroergotamine, pronethalol, guanethidine bretylium, reserpine, atropine and papaverine on transmurally stimulated guinea-pig ileum was antagonized by tolazoline.

3. The potentiating effect of tolazoline on transmurally stimulated ileum persisted after blockade of ganglionic cells by hexamethonium, but disappeared after 30 min exposure of the ileum to hemicholinium or procaine.

4. Tolazoline raised the tonus and potentiated the responses of isolated guinea-pig ileum to acetylcholine, potassium chloride, barium chloride, 5-hydroxytryptamine and angiotensin. The stimulatory effect of tolazoline disappeared after the addition of atropine.

5. Higher doses of tolazoline reduced responses to acetylcholine and histamine. However, the antihistaminic drugs antazoline and promethazine failed to reduce the stimulatory effect of tolazoline on the electrically stimulated guinea-pig ileum.

6. On depolarized guinea-pig ileum preparation, tolazoline failed to potentiate the action of acetylcholine.

7. The potentiating effect of tolazoline on the guinea-pig ileum is discussed in relation to its anticholinesterase action.

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