THE ROLE OF HISTAMINE IN URETERAL FUNCTION[1, 2]

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Stimulation of the ureter by exogenous histamine *in vitro*, first observed by Rothmann (1927) has been confirmed by Gruber (1928), Binet and Seringe (1936) and Chen *et al.* (1957). In a study of action potentials *in situ*, Sleator and Butcher (1955) also showed that histamine, after local or systemic administration, has a stimulatory effect on the dog ureter. Conflicting observations have been reported by Czok and Kreienberg (1951) and by Thackston *et al.* (1955), who failed to observe any stimulatory or other effect of histamine on the ureters of pigs, rats and rabbits. The histamine-blocking effects of antihistamines on the ureters of dogs and pigs *in vitro* were first studied by Frignani and Pezzuoli (1957), who also found that some antihistamines stimulated the ureter. Their findings were largely confirmed by Chen *et al.* (1957).

The present study was undertaken to investigate further the reaction of the ureter to exogenous histamine, and to evaluate factors which influence its reactivity with a view to the possibility, suggested by Sleator and Butcher (1955) and by Chen *et al.* (1957), that histamine or a related agent may play a physiological role in normal ureteral activity. This view is based primarily on the well-established fact that, of agents which occur in the body under normal conditions and whose effects on the ureter have been studied under a variety of conditions, histamine is the most consistent stimulant.

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[2] We wish to express our appreciation to the following firms for the generous supply of several drugs used in the experiments: Abbott Laboratories, North Chicago, Illinois, for serotonin; Ciba Pharmaceutical Products, Inc., Summit, New Jersey, for tripelennamine; Parke, Davis & Co., Detroit, Michigan, for diphenhydramine; Sandoz Pharmaceuticals, Hanover, New Jersey, for LSD-25.

METHODS. The ureters used in these experiments were removed from apparently healthy adult mongrel dogs of either sex under pentobarbital sodium anesthesia (30 mg/kg intravenously). The specimens were immediately placed in a modified preoxygenated Tyrode-Locke solution at 4°C and stored at this temperature until used. The Tyrode's solution was saturated with a gas mixture of 95% O_2 and 5% CO_2 and had a pH of 7.4. Its composition was as follows: NaCl, 137 mM; KCl, 2.7 mM; $CaCl_2 \cdot 2H_2O$, 1.8 mM; $MgCl_2 \cdot 6H_2O$, 1.05 mM; $NaHCO_3$, 24 mM; $NaH_2PO_4 \cdot H_2O$, 0.21 mM. Dextrose 10 mM was added when this solution was used as bath fluid but was omitted for refrigerated storage. The bath used in the present study was similar to that described by Trendelenburg (1913) for studying the peristaltic reflex in the gut. The ureteral segments were tied to a concentric double-barreled cannula (the outer barrel was a blunt No. 13 needle, the inner barrel a blunt No. 18 needle). The ureteral segments were 2.5 cm long, a length chosen because it corresponds to that of a ureteral contraction wave *in vivo*. The segments were thus able to contract more or less simultaneously throughout their entire length.

The segments were perfused slowly by means of a motor-driven syringe pump, with the inflow through the inner barrel and outflow through the outer one. A manometer was connected to the outflow line to record perfusion pressure, a record of which is important, since our results showed that intraluminal pressure is often the determining factor for eliciting mechanical activity in the specimens. A perfusion pressure of 25 cm of water was chosen because it gave the most consistent results, and did not produce damage by overdistention. The perfusion rate was adjusted to between 4 and 5 ml/min for the same reasons. The relation between intraluminal pressure and the initiation of the first contraction has been the subject of a separate study (Atwell and Borgstedt, 1960).

The upper ends of the ureteral segments were ligated and attached to a simple lever with a

mechanical amplification of the longitudinal contractions of 11 to 1 and a tension on the ureter of about 2.5 g. The bath was maintained at 38°C and oxygenated with 95% O_2 5% CO_2. The temperature of 38°C is apparently not critical; in the range from 36°C to 39°C no changes were observed in the behavior of the ureters, although at temperatures above 40°C irregular contractions occurred. This effect has been used on occasion to produce activity in otherwise quiescent ureters (Primbs, 1913). Following a suggestion by Macht (1916), 0.2% of urea added to the bath fluid in some preliminary experiments markedly increased the tendency of the specimens for mechanical activity in the absence of perfusion.

Ureteral segments freshly installed in the bath chamber usually contracted irregularly for about 10 to 15 minutes whether perfused or not; but after this initial period of stabilization most of the specimens became quiescent in the absence of perfusion and did not show longitudinal contractions in response to bursts of perfusion. Such segments were used in this study to test the effects of histamine and other stimulatory agents. Similar contractions in unstabilized preparations have also been reported by Sokoloff and Luchsinger (1881), Stern (1903) and Zanne (1937). About 20% of the specimens, however, did not become quiescent at any time during the experiment and persistently contracted in response to perfusion. In 3 experiments, contractions persisted even in the absence of perfusion. There is, as yet, no apparent explanation for these variations, and no correlation with sex, region of the ureter from which the specimen was prepared or with any of the experimental conditions under our control. These segments afforded an opportunity to compare for certain drugs the effects on spontaneous activity with the effects on pharmacologically induced activity.

All drugs were freshly prepared in aqueous solution. The concentrations were so adjusted that no more than 2 ml of drug solution were added to the bath fluid (100 ml) or to the same volume of perfusate. The drugs were used in the following forms: Acetylcholine chloride, dihydroergotamine methanesulfonate, diphenhydramine hydrochloride, epinephrine chloride, histamine diphosphate, lysergic acid diethylamide tartrate, norepinephrine bitartrate, serotonin creatinine sulfate and tripelennamine hydrochloride.

RESULTS. The results were obtained on ureters from 22 dogs, 12 male and 10 female. Two or three segments were studied from each ureter, making a total of 68 experiments.

Histamine. After the initial period of stabilization about 80% of the examined specimens became quiescent and showed no peristaltic activity in response to increases in intraluminal pressure associated with perfusion (see initial period in fig. 1 part A). When histamine was added to the bath fluid in concentrations of 0.4 to 0.6 $\times 10^{-5}$ M, the segments remained inactive in the absence of perfusion; but when perfused they responded with a brief burst of peristaltic activity. As the concentration of histamine was increased stepwise to 1.2 $\times 10^{-5}$ M, the peristaltic response increased gradually in duration, but not in amplitude or frequency. This dose-response relationship is illustrated in figure 1A and B. With a histamine concentration of 1.2 $\times 10^{-5}$ M, activity persisted throughout the perfusion period but ceased when perfusion was stopped (see final 3 perfusion periods in fig. 1B).

Concentrations higher than about 1.4 $\times 10^{-5}$ M produced a change in the character of the response (see fig. 2B). The frequency of the contractions was increased considerably while their amplitude was decreased. During the short periods of relaxation between the frequent contractions the baseline remained at a considerably higher level than observed with the less frequent contractions produced by lower concentrations of histamine. This sustained "tetanic" or "spastic" activity persisted for only a few minutes, during which the amplitude of the contractions decreased even further, followed by an ultimate cessation of ureteral activity and a return of the baseline to a level near that existing before the period of perfusion. This latter effect would occur after the recording in figure 2C and is not illustrated.

Also, beginning at concentrations of about 1.2 $\times 10^{-5}$ M, some segments showed contractions even in the absence of perfusion; the frequency of these contractions tended to increase with increasing concentrations of histamine.

The effect of histamine was persistent. In 5 of 6 segments whose responsiveness to perfusion had been established by a sufficient concentration of histamine in the bath fluid, activity could be elicited by perfusions at intervals for at least 30 minutes without more than a 10% decrease in amplitude or frequency. In the sixth segment so tested, activity was induced by histamine even in the absence of perfusion (similar to the initial period in fig. 4) and the amplitude of the contractions decreased rapidly to zero in about 15

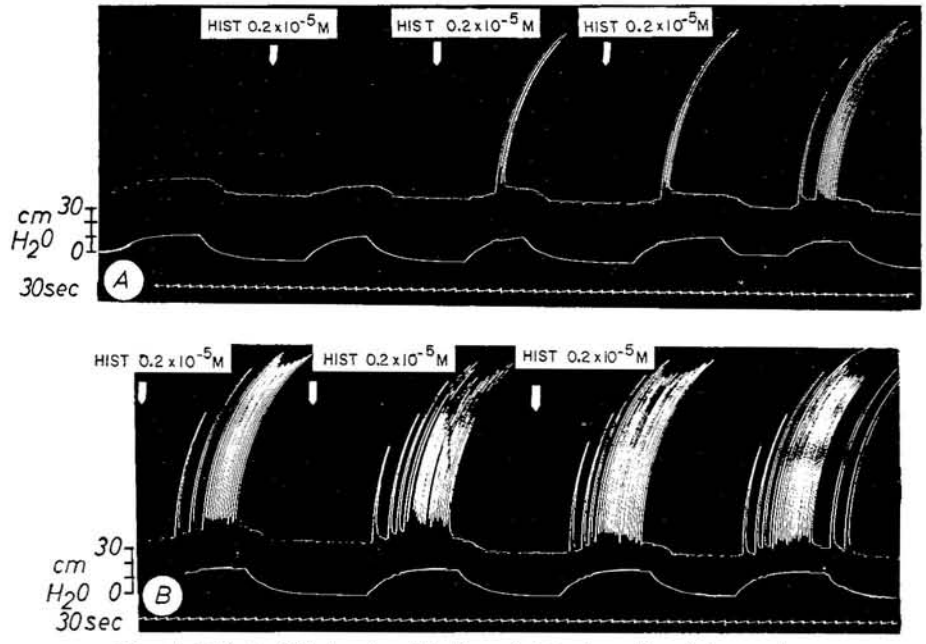

FIG. 1. Effect of histamine (Hist) on isolated, perfused dog ureter.
In each panel, upper tracing: isotonic contraction of longitudinal muscle; middle tracing: beginning and end of perfusion; lower tracing: time. Histamine (0.2×10^{-5} M) was added between periods of perfusion (additions cumulative—no washing). B is a direct continuation of A.

minutes, indicating exhaustion of the preparation. Since activity produced by these low concentrations of histamine could usually be terminated by washing, it can be concluded that exogenous histamine persists in the bath fluid for at least 30 minutes, although no attempt was made to measure its possible partial destruction. Although it was usually possible to abolish the activity induced by the minimal stimulatory concentration of histamine, when higher concentrations of histamine had been employed some residual activity tended to persist even after repeated (up to 6 times) changes of the bath solution.

When histamine was added to the fluid passing through the lumen of the ureteral segments during periods of perfusion so that it came initially into contact with the mucosal surface, the minimal stimulatory concentrations were about 10 times greater than those necessary to produce stimulation when added to the bath fluid (fig. 2). Ureters stored under refrigeration at 4°C (up to 11 days) in Ringer solution remained responsive to histamine (fig. 3). Such stored specimens showed a response to the same concentrations of histamine as freshly prepared segments although the amplitude of the contractions was decreased and their regularity frequently disturbed.

Interactions of histamine with other drugs. In these experiments contractions were always induced by adding histamine in steps of 0.2×10^{-5} M until the minimal stimulatory concentration (0.4 to 0.6×10^{-5} M) was reached. Drugs were added to the bath fluid in the presence or absence of histamine.

The drugs and concentrations used are listed in table 1 together with a summary of their effects: a) on histamine-induced contractions, b) on spontaneous contractions, and c) on quiescent segments. Adrenergic compounds (epinephrine and norepinephrine) had some stimulating action, whereas acetylcholine had no effect whatever. It is important to note that even in the relatively high concentrations used none of these agents had an inhibitory effect on either spontaneous (see fig. 5B and C) or histamine-induced activity of the ureter. Only the antihistamines, which are discussed below, had a strongly inhibitory action

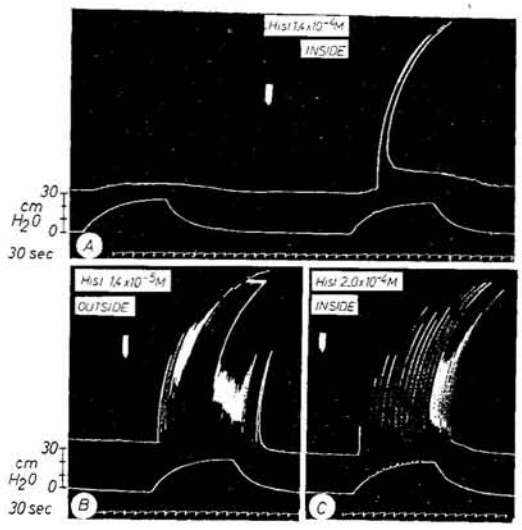

FIG. 2. Effect of histamine (Hist) on isolated, perfused dog ureter.
A: 1.4×10^{-4} M of Hist added to perfusate (inside) produced a threshold response (consisting of 3 "grouped" contractions). B: 1.4×10^{-5} M Hist added to the bath fluid (outside) produced a "spastic" response in the same preparation (frequency higher than 25 contractions per minute, upper excursion of lever at height of response limited mechanically). C: 2.0×10^{-4} M Hist added again to the perfusate (inside), produced regular sustained contractions (frequency at first 4 per minute, changing to 17 per minute).

on histamine-induced activity (see also Borgstedt[3]).

Antihistamines. Activity produced by concentrations of histamine just sufficient to show their effect could be abolished by diphenhydramine and tripelennamine in concentrations between 0.1 and 0.2 $\times 10^{-5}$ M (fig. 4A). The addition of larger amounts of histamine (about 1.6×10^{-5} M) caused the reappearance of ureteral activity even in the presence of these concentrations of antihistamines (fig. 4B). The stimulatory actions of relatively high concentrations of histamine (2.0×10^{-5} M) could not be abolished completely by antihistamines in any concentration up to 5×10^{-5} M. Moreover, these concentrations of antihistamines, with the exception of tripelennamine, depressed ureteral activity of any kind including spontaneous activity and that produced by barium chloride. Since the concentrations of antihistamines required to abolish spontaneous contractions are much higher than those effective against histamine-induced ones (see fig. 5A), it could be inferred that histamine is not involved in the contractile mechanism itself under our experimental conditions. Tripelennamine alone in concentrations over 2.0×10^{-5} M showed some stimulatory effects. This, according to Frignani and Pezzuoli (1957), is a common property of antihistamines built around the diaminoalkyl configuration. It is noteworthy that concentrations of diphenhydramine which abolished histamine-induced contractions did not suppress spontaneous contractions (fig. 5), neither did this drug stimulate the ureter.

[3] Abstract of a paper by H. H. Borgstedt presented at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, August 1958, Ann Arbor, Michigan.

DISCUSSION. The observations here reported appear to be best interpreted as indicating that the site of action of histamine on the ureter is the smooth muscle cell itself. This is strongly suggested by the fact that the effects of histamine are essentially the same in ureteral segments stored up to 11 days. Both the regular peristaltic response and the "spastic" or "tetanic" response could be observed after this period of time (see fig. 3C). The reported presence of scattered ganglion cells (Protopopow, 1897) and scattered ganglia of some 100 to 200 cells in the adventitia or the outer longitudinal layer of the ureter near the bladder (Waldeyer's sheath), is still a controversial matter. In more recent investigations Kantner (1957) and Bergman (1958) described only nerve fibers of various types without special end apparatus, never ganglion cells. These facts alone make it seem unlikely that histamine exerts its effect *via* ganglionic elements. But even if ganglion cells and fibers were present, a possibility which cannot as yet be completely excluded, it seems unlikely that these cells would retain their responsiveness for as long as 11 days of refrigerated storage. Stave (1954), in his work on the isolated taenia coli of the guinea pig, came to similar conclusions. After about 2 days of storage his preparations lost their tonic functions and a, presumably myogenic, automatic rhythmicity appeared which persisted up to 11 days. After the first 2 days his colon preparations behaved pharmacologically similarly to our ureteral preparations. The specific site in or on the smooth muscle cell at which histamine might act is not clear at present. Bergman (1958) has described certain myocytal interjunctions with a specialized structure in his electronmicrographs of ureteral

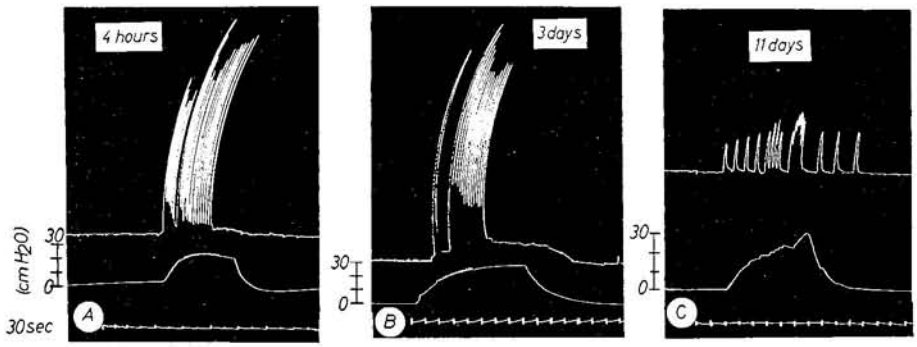

FIG. 3. Effect of histamine (Hist) on perfused dog ureter at different times after isolation. All effects after addition of 0.6×10^{-5} M Hist to the bath fluid. A: Vigorous sustained response 4 hours after removal (frequency 13 per minute). B: Essentially the same response 3 days after removal (frequency of 12 per minute). C: 11 days after removal amplitude and frequency (average 4 per minute) are diminished. A, B and C represent experiments on 3 segments of the same ureter. Note period of "spastic" response in C.

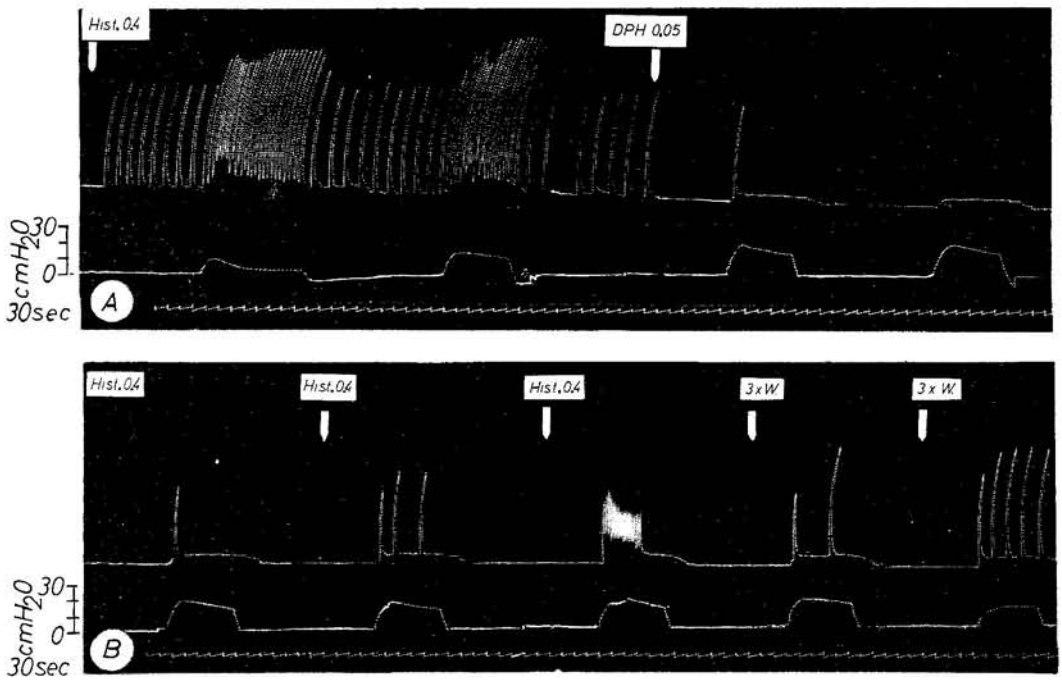

FIG. 4. Effects of histamine (Hist) and diphenhydramine (DPH) on isolated perfused dog ureter. Concentrations noted are all $\times 10^{-5}$ M. B is a direct continuation of A. A: Hist (0.4×10^{-5} M) causes threshold response (note in this experiment contractions at rate of 2.5/min between periods of perfusion). DPH (0.05×10^{-5} M) promptly abolished contractions between periods of perfusion and those in response to perfusion during the subsequent period. B: Previously established concentration of Hist (A) caused 1 contraction; doubling the concentration (additions cumulative without washing) caused 3; trebling caused sustained response (rate of 18 per min). This response could not be completely abolished by washing repeatedly $3 \times W = 3$ changes of the bath fluid in rapid succession.

muscle. He suggested that these may be the site of a transmitter mechanism and proposed histamine as an agent.

Spontaneous contractions of ureteral segments could not be abolished by concentrations of diphenhydramine comparable to those effective against contractions induced by histamine. This makes it appear unlikely that spontaneous contractility is dependent merely upon the extracellular presence or release of histamine.

TABLE 1

Effects of drugs on isolated, perfused dog ureter

Drug	Concentrations	No. of Preps	Spontaneous Contractions	Histamine-Induced Contractions	Quiescent Ureter
Acetylcholine	10^{-7}–10^{-4} M	6	No effect	No effect	No effect
Atropine	10^{-5}–10^{-3} M	5	No effect	No effect	No effect
Epinephrine	10^{-6}–10^{-4} M	8	No effect	Inconsistent increase in frequency	No effect
Norepinephrine	10^{-6}–10^{-4} M	6	Inconsistent increase in frequency	Inconsistent increase in frequency	Inconsistent stimulation (*ca.* 20%)
Dihydroergotamine	10^{-6}–10^{-4} M	5	No effect	No effect	No effect
Serotonin	10^{-9}–10^{-5} M	5	No effect	—	No effect
Lysergic acid diethylamide	10^{-9}–10^{-7} M	6	Increase in frequency	Some increase in frequency	—

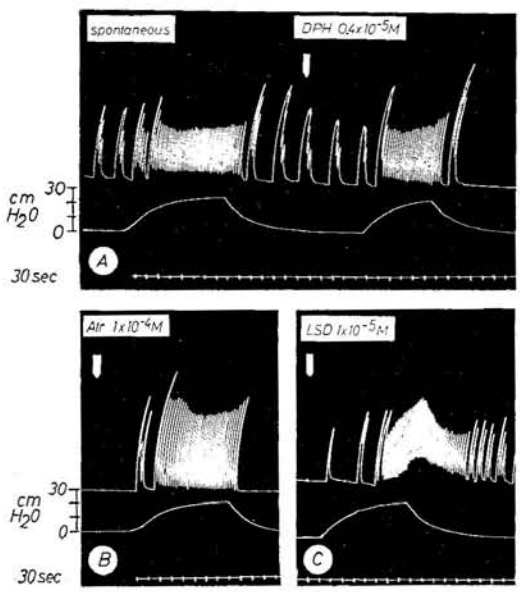

FIG. 5. Effect of diphenhydramine (DPH), atropine (Atr) and lysergic acid diethylamide (LSD) on isolated perfused dog ureter.

A: Spontaneous contractions between and during periods of perfusion were not abolished by 0.4×10^{-5} M DPH, a much higher concentration than that effective against histamine-induced contractions (see fig. 4). B: Atr (1×10^{-5} M) had no effect on contractions. C: LSD (1×10^{-5} M) also had no effect (recording pens for contractions and pressure were misaligned, contractions occurred simultaneously with increase in pressure).

Since the concentrations of antihistamines effective against spontaneous contractions are much higher than those effective against histamine-induced ones it is probable that exogenous histamine also is not involved directly in the contractile mechanism responsible for peristaltic activity.

The fact that higher concentrations of histamine were necessary to produce an effect when administered from the mucosal side speaks against the presence of specific receptors in the ureteral mucosa, a possibility which has to be taken into account in view of the recent discovery by Bülbring and Lin (1958) of serotonin- and pressure-sensitive receptors in the mucosa of the guinea-pig ileum.

An important factor influencing the responses of the ureter to drugs is the mechanical load, produced in our experiments by perfusion under controlled pressure simulating the physiological conditions. In the present studies perfused ureters responded to lower concentrations of histamine than did nonperfused ones. The latter usually showed no regular contractions and, in most instances, no contractions at all except with relatively high concentrations of histamine. In relatively low concentrations histamine thus has a facilitatory effect on what may be called the "peristaltic reflex" of the ureter: in fact, in most of our experiments the presence of histamine was necessary to enable the elicitation of this response. This effect, however, is not peculiar for histamine alone. It can also be observed with other consistently stimulatory agents such as barium chloride and lysergic acid diethylamide. The lead toward a better understanding of ureteral contractility and rhythmicity perhaps will lie in an explanation of how mechanical factors influence the manifestation of drug effects in this system.

SUMMARY

Under properly controlled conditions of perfusion and pressure *in vitro*, histamine has a consistently stimulatory effect on canine ureteral segments. Inability of some investigators to obtain such stimulation may be ascribed to the determining role which mechanical factors play in influencing sensitivity and the type of response to histamine.

The effects of histamine appear to be most readily interpreted as facilitation of the peristaltic reflex of the ureter by an action directly on the smooth muscle cells but not on the contractile mechanism itself. This, however, does not preclude the possibility that histamine plays a role in the normal function of the ureter.

The effects produced by histamine, under certain conditions, can be abolished by antihistamines in concentrations which do not inhibit spontaneous contractions.

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