amount of intact bumetanide present. This finding suggests that either no appreciable levels of metabolites were present or that bumetanide was selectively extracted by ether. In any event, the ether extraction step in the radioimmunoassay procedure should ensure that adequate specificity is obtained.

Although the major reason for the development of a radioimmunoassay for bumetanide was to obtain high sensitivity, the radioimmunoassay, due to its simplicity, allows a large number of samples to be assayed with relative ease compared to GC and spectrofluorometric methods.

In humans, the major portion of bumetanide excreted in the urine is excreted at the times when the plasma levels of bumetanide are at their peaks. These results suggest that the amount of excretion is proportional to the plasma level of bumetanide. This conclusion is further supported by the finding that the apparent half-lives calculated from either the urinary excretion data or the plasma level data are essentially the same.

Davies *et al.* (2) found that 47% of a 1-mg intravenous dose of ^{14}C-bumetanide was excreted in the urine within 3 days and that 16% was excreted in the feces within 8 days. Following the 2-mg oral dose of bumetanide to the eight subjects in this study, a mean of 43% of the dose was excreted in the urine during the 1st day, suggesting that these oral doses were completely absorbed. In addition, the mean renal clearance of bumetanide of 102 ml/min following intravenous administration to normal subjects (2) is in excellent agreement with the renal clearance of 107 ml/min found in the present study following oral administration. These results further suggest complete absorption of orally administered drug.

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ACKNOWLEDGMENTS AND ADDRESSES

Received April 30, 1975, from the *Department of Biochemistry and Drug Metabolism and the Chemical Research Division, Hoffmann-La Roche Inc., Nutley, NJ 07110*

Accepted for publication July 28, 1975.

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Muscle Relaxant Properties of Chloramphenicol

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Abstract □ Experiments with the guinea pig ileum, guinea pig trachea, rat fundal strip, rat colon, rat vas deferens, and toad heart indicated that chloramphenicol inhibited smooth muscles, decreasing both the height and frequency of spontaneous contraction. Chloramphenicol-induced relaxation was not mediated through adrenergic, cholinergic, or histaminergic mechanisms. The degree of muscle relaxation was related to the concentration of chloramphenicol, and the relaxant effect could be reversed by removing chloramphenicol from the site of action by washing. Its action appears to be direct on the muscle, possibly by interfering with the energy-generating mechanism.

Keyphrases □ Chloramphenicol—effect on smooth muscle relaxation, guinea pig □ Muscle relaxant activity—chloramphenicol, effect on smooth muscles, guinea pig □ Antibacterials—chloramphenicol, effect on smooth muscle relaxation, guinea pig

Chloramphenicol depresses the spontaneous movements of the rabbit ileum (1). It antagonizes the histamine- or acetylcholine-induced response of the rabbit ileum (1) and the dog bronchial chains (2). Chloramphenicol is 15–25 times more active than ephedrine in this respect (2).

This study was concerned with examining the muscle relaxant properties of chloramphenicol to understand its mode of action. Therefore, the effect of chloramphenicol was determined on different tissues containing smooth muscle such as the guinea pig ileum, rat fundal strip, rat colon, and rat uterus and in isolated organ systems representing the adrenergic, cholinergic, and histaminergic systems. The perfused toad heart, rabbit aortic strip, and rabbit ileum preparations were selected to examine the effect of chloramphenicol on the adrenergic system.

The perfused toad heart preparation exemplified a muscle system containing β-adrenergic receptors with strong autogenous contractile drive, whereas the aortic strip and rabbit ileum preparations represented muscle systems innervated with α-adrenergic receptors. The rabbit jejunum, having a muscle contractile system with a moderate autogenous drive, served as the model of the cholinergic system. The guinea pig trachea, having a muscle system with a weak autogenous contractile drive, served as the representative histaminergic system (3).

EXPERIMENTAL

Toad Heart Perfusion (Adrenergic System)—Toads (*Bufo melanostictus*) of either sex, 75–80 g, were pithed. The abdomen was opened by a midline incision, the pericardium was removed, and a venous cannula was inserted into one hepatic vein opening into the sinus venosus for perfusion with a Symes cannula. The perfusion fluid was frog–Ringer solution. A fine hook was fixed at the tip of the ventricle and was connected through a silk thread with an isotonic lever.

The drugs were injected into the rubber tube attached to the cannula to determine their effect on the spontaneously beating heart. The heart perfusion was continued for 30 min to attain equilibrium before any recordings were taken. For each experiment,

the number of beats was counted for the first 2 min to obtain the frequency.

Rabbit Aortic Strips (Adrenergic System)—Albino rabbits, 1.5–3 kg, were decapitated after being stunned. The descending thoracic aorta was removed and placed in Krebs–Hensleit solution, and excess fat and connective tissue were removed. The aorta was cut along a closed spiral to form a continuous spiral strip from which small aortic strips, 0.4 mm thick, 2 mm wide, and 2.4 cm long, were prepared (4, 5). One end of the aortic strip was fixed at the base of the tissue chamber, having a volume of 30 ml and containing Krebs–Hensleit solution; the upper end was attached to an isotonic lever.

The lever was adjusted to provide an amplification of nine times and counterweighted to impart a tension of 4 g on the strip. The bath fluid was saturated with a mixture of 95% oxygen and 5% carbon dioxide and maintained at 37.5°. Under 4 g of tension, the strips immediately stretched about 40%, followed by a further stretch of 2–10% during the next 10–60 min. Because of this elongation, the strips were allowed to equilibrate for 2–3 hr before the effects of the drugs were studied.

Rabbit Ileum (Adrenergic System)—Albino rabbits were killed as already described. A section of 4 cm of ileum was excised through a midline incision on the abdomen. The contents of the intestine were removed by gentle washing of the lumen with Tyrode solution kept at 37.5°. A section of approximately 2–2.5 cm of ileum was suspended in a 30-ml organ bath containing Tyrode solution saturated with 95% oxygen and 5% carbon dioxide and maintained at 37.5°. The tissue was equilibrated for 30 min in the organ bath with frequent washings before any contraction was recorded.

Rabbit Jejunum (Cholinergic System)—The preparation was the same as that described for the rabbit ileum. A section of 4 cm of jejunum was excised, 20–30 cm posterior to the pylorus.

Guinea Pig Tracheal Chain (Histaminergic System)—Guinea pigs, 200–250 g, were killed by a blow on the head. The trachea was removed, washed with warm Krebs–Hensleit solution, and then sectioned into 12 rings of about the same width. The rings were connected in series by means of short loops of silk thread in such a way that the dorsal smooth muscle band was vertical in the chamber (6). The chain was suspended in the organ bath at 37.5°; the bath contained Krebs–Hensleit solution saturated with 95% oxygen and 5% carbon dioxide.

A light, sensitive isotonic writing lever with 12-fold magnification was used to record contraction or dilatation of the tracheal chain. The tracheal chain was equilibrated for 60–90 min in the organ bath with frequent washings prior to the recordings.

Other Preparations—Studies also were undertaken with the guinea pig ileum, rat fundal strip, rat colon, rat vas deferens, and rat estrous uterus preparation (7), with acetylcholine, histamine, 5-hydroxytryptamine, nicotine, and pituitrin being used as agonists. The organ bath and the recording device were the same as those described earlier.

Chloramphenicol and other drugs were dissolved or diluted in isotonic sodium chloride solution of pH 7 and added to the bath fluid in the least volume possible to produce minimal osmotic shock to the tissues.

RESULTS

Effect of Chloramphenicol on Isolated Guinea Pig Ileum—The agonists used were acetylcholine, histamine, 5-hydroxytryptamine, and nicotine. Significant alteration in the height of contraction of guinea pig ileum was observed only after relatively high doses of chloramphenicol. Changes in the height of contraction became evident at a concentration of 10 μg/ml of bath for acetylcholine, 20 μg/ml for 5-hydroxytryptamine and nicotine, and 40 μg/ml for histamine.

Incremental increases in concentrations of chloramphenicol from 10 to 320 μg/ml for agonist acetylcholine, from 40 to 640 μg/ml for agonist histamine, and from 20 to 320 μg/ml for agonist nicotine and 5-hydroxytryptamine produced progressive reductions in the height of contraction by the guinea pig ileum (Table I).

Effect of Chloramphenicol on Rat Fundal Strip—The agonist used was 5-hydroxytryptamine. Alterations in the height of contraction of the fundal strip were observed at a relatively high concentration of chloramphenicol (250 μg/ml of bath). Incremental increases in the concentration of chloramphenicol from 250 μg/ml to 2 mg/ml produced a linear reduction in the height of contraction (Table I).

Table I—Chloramphenicol-Induced Inhibition of Smooth Muscles Using Various Agonists

Tissue Preparation	Agonist, μg/ml Bath	Chloramphenicol, μg/ml Bath	Inhibition Compared to Agonist-Induced Contraction, %, Mean ± *SE*
Guinea pig ileum	Acetylcholine, 0.66	10	17 ± 2
		20	24 ± 3
		40	31 ± 4
		80	54 ± 8
		160	83 ± 2
		320	91 ± 2
	Histamine, 0.0031	40	11 ± 4
		80	25 ± 4
		160	48 ± 7
		320	78 ± 6
		640	90 ± 4
	5-Hydroxytryptamine, 0.43	20	25 ± 7
		40	28 ± 4
		80	44 ± 7
		160	64 ± 13
		320	87 ± 2
	Nicotine, 0.33	20	19 ± 6
		80	51 ± 6
		80	51 ± 12
		160	71 ± 8
		320	90 ± 4
Rat fundus strip	5-Hydroxytryptamine, 0.23	250	32 ± 5
		500	49 ± 4
		1000	72 ± 5
		2000	89 ± 4
Rat colon	5-Hydroxytryptamine, 0.1	10	10 ± 5
		20	20 ± 5
		20	29 ± 4
		80	40 ± 3
		160	62 ± 4
		320	80 ± 3
	Acetylcholine, 0.033	50	32 ± 2
		100	50 ± 1
		200	72 ± 4
		400	90 ± 4
Rat estrous uterus	Pituitrin, 0.033	20	6 ± 2
		40	14 ± 2
		80	24 ± 4
		160	50 ± 10
		320	92 ± 4
	5-Hydroxytryptamine, 0.008	2	15 ± 5
		4	18 ± 5
		8	14 ± 6
		16	24 ± 6
		32	46 ± 1
		64	93 ± 2
Rat vas deferns	Epinephrine, 2.0	160	17 ± 8
		320	26 ± 6
		640	37 ± 2
		1280	54 ± 5
		2560	81 ± 3

Effect of Chloramphenicol on Rat Colon—Alteration in the height of contraction of the rat colon was observed with 10 μg of chloramphenicol/ml of bath for the agonist 5-hydroxytryptamine and 50 μg of chloramphenicol/ml for the agonist acetylcholine. Incremental increases in chloramphenicol concentrations from 10 to 320 μg/ml for 5-hydroxytryptamine and from 50 to 400 μg/ml for acetylcholine produced a progressive reduction in the height of rat colon contraction (Table I).

Effect of Chloramphenicol on Rat Estrous Uterus—The agonists used were 5-hydroxytryptamine and pituitrin. Alterations in the height of contraction were observed with a concentration of chloramphenicol of 2 μg/ml of bath for the agonist 5-hydroxytryptamine and 20 μg/ml for pituitrin. Incremental increases in the concentration of chloramphenicol from 2 to 64 μg/ml for the agonist 5-hydroxytryptamine and from 20 to 320 μg/ml for pituitrin produced a progressive reduction in the height of contraction (Table I).

Effect of Chloramphenicol on Rat Vas Deferens—The agonist used was epinephrine. Alterations in the height of contraction

Table II—Chloramphenicol-Induced Inhibition of Rate (Frequency) and Height (Amplitude) of Contraction of Toad Heart

Dose of Chloramphenicol, mg	Inhibition, %[a]	
	Frequency per Minute	Amplitude, mm
0.25	6 ± 1	27 ± 5
0.5	3 ± 1	29 ± 5
1.0	4 ± 1	51 ± 2
2.0	4 ± 1	81 ± 4
4.0	15 ± 6	97 ± 2
8.0	68 ± 6	100 (total)

[a] Mean of seven observations ± *SE*.

were observed with a relatively high concentration of chloramphenicol, 160 μg/ml of bath. Incremental increases in the concentration of chloramphenicol from 160 to 2560 μg/ml produced a progressive reduction in the height of contraction (Table I).

Effect of Chloramphenicol on Perfused Toad Heart—Reductions in the rate and height of contraction of the spontaneously beating toad heart were observed only after a relatively high dose of chloramphenicol, 0.25 mg. Incremental increases in the dose of chloramphenicol from 0.25 to 8 mg produced a progressive reduction in the amplitude of contraction. The frequency of the beat, however, changed significantly only when the dose was increased to 4 mg. Higher concentrations produced a state of contracture of the heart (Table II).

To determine the efficacy of chloramphenicol on the reduction of force and rate of contraction of the heart, the agonists epinephrine and histamine were selected. Under controlled conditions, 3 mg of chloramphenicol produced an average reduction of the frequency by five beats/min and of amplitude by 57 mm. When the heart was stimulated with 0.2 μg of epinephrine, 3 mg of chloramphenicol produced an average reduction of the frequency by 13 beats/min and of amplitude by 42 mm. Similarly, after the heart was stimulated with 10 μg of histamine, the same dose of chloramphenicol led to an average reduction of frequency by 42 beats/min and of amplitude by 54 mm (Table III).

To ascertain that the action of chloramphenicol was direct and not mediated through inhibition or release of mediators, the effects of several blocking agents were studied. The efficacy of the block was tested by challenging the tissue with the appropriate agonist. After the addition of 0.2 μg of propranolol hydrochloride/ml in the perfusion fluid, no significant changes in frequency and amplitude from the control values were observed. The addition of 3 mg of chloramphenicol in the perfusion fluid in the presence of the challenger epinephrine and propranolol produced an average reduction of frequency of the heart beat by 21 beats/min and of amplitude by 47 mm below that of propranolol alone (Table III).

The addition of 0.25 μg of atropine sulfate/ml in the perfusion fluid did not significantly change either the frequency or the amplitude of the heart beat in comparison to the control values. After the adequacy of the atropine block was ascertained with 0.04 μg of acetylcholine, 3.3 mg of chloramphenicol produced an average reduction of 31 beats/min and a 48-mm reduction in the amplitude of contraction (Table III).

Physostigmine, 4.7 μg/ml in the perfusion fluid, did not significantly change either the frequency or the amplitude of the heart beat in comparison to the control values. The addition of the standard dose of 3.4 mg of chloramphenicol, in the presence of physostigmine in the perfusion fluid, reduced the frequency by 14 beats/min and the amplitude of contraction by 52 mm as compared to the control values (Table III).

Effect of Chloramphenicol on Isolated Rabbit Aortic Strip—The minimum amount of chloramphenicol required to produce relaxation in the isolated rabbit aortic strip was 0.5 mg/ml of bath. Increasing the concentration of chloramphenicol from 0.5 to 4 mg/ml produced a progressive reduction in the height of contraction by the aortic strip. The addition of an average dose of 16 μg of epinephrine/ml produced a sustained contraction of the aortic strip, which was found to be antagonized minimally by 0.5 mg and maximally by 4 mg of chloramphenicol/ml. The presence of chloramphenicol in the bath before the addition of epinephrine diminished the epinephrine response; the mean antagonism was 15, 31, 47, and 74% for 0.5, 1, 2, and 4 mg of chloramphenicol/ml, respectively. Chloramphenicol behaved as an adrenergic negator by inhibiting the epinephrine response (Table IV).

Epinephrine stimulation, 9 μg/ml, increased the force of contraction, which was inhibited by about 60% by the addition of phentolamine hydrochloride, 0.5 μg/ml of bath. Addition of 4 mg of chloramphenicol/ml to this preparation further reduced the height of contraction by about 89% (Table IV).

Effect of Chloramphenicol on Isolated Rabbit Ileum—Epinephrine, 0.267 μg/ml of bath, produced maximal relaxation. Increase in the concentration of epinephrine to 0.533 μg/ml diminished the relaxation response. Phentolamine hydrochloride was added to the bath, 2.5 μg/ml, and then epinephrine, 0.267 μg/ml,

Table III—Effect of Chloramphenicol on the Perfused Toad Heart in the Presence of Various Agonists and Blocking Agents[a]

Control	Effect of Chloramphenicol	Agonist or Blocker Used	Effect of Agonist or Blocker	Combined Effect of Agonist or Blocker plus Chloramphenicol
		Heart Beats per Minute		
63 ± 3	58 ± 5 (3 mg)	Epinephrine (0.2 μg)	71 ± 3	58 ± 6
63 ± 3	58 ± 5 (3 mg)	Histamine (10 μg)	68 ± 2	26 ± 5
58 ± 5	42 ± 9 (3 mg)	Propranolol (0.2 μg/ml)	54 ± 4	33 ± 8
65 ± 6	45 ± 7 (3.3 mg)	Atropine (0.25 μg/ml)	62 ± 5	31 ± 8
72 ± 6	58 ± 6 (3.4 mg)	Phyostigmine (4.7 μg/ml)	67 ± 5	53 ± 5
		Height of Contraction, mm		
67 ± 4	10 ± 2 (3 mg)	Epinephrine (0.2 μg)	80 ± 2	38 ± 8
67 ± 4	10 ± 2 (3 mg)	Histamine (10 μg)	54 ± 3	0 (complete inhibition)
51 ± 5	8 ± 3 (3 mg)	Propranolol (0.2 μg/ml)	49 ± 5	2 ± 1
50 ± 6	2 ± 0.5 (3.3 mg)	Atropine (0.25 μg/ml)	51 ± 6	3 ± 2
67 ± 4	10 ± 3 (3.4 mg)	Physostigmine (4.7 μg/ml)	60 ± 4	8 ± 3

[a] Values are means of seven observations ± *SE*. Figures in parentheses indicate the dose of the drug used. The dose is total dose in the case of chloramphenicol and agonists and per milliliter of perfusion fluid in the case of blocking agents. Differences in the values in columns 1 and 4 and in columns 2 and 5 are not statistically significant.

Table IV—Effect of Chloramphenicol on the Height of Contraction of the Isolated Rabbit Aortic Strip in the Presence of Agonists and Blocking Agents

Drug Used	Height of Contraction of Aortic Strip[a], mm
Epinephrine (16 μg/ml of bath)	43 ± 1
plus chloramphenicol (0.5 mg/ml)	34 ± 3
plus chloramphenicol (1 mg/ml)	29 ± 4
plus chloramphenicol (2 mg/ml)	23 ± 4
plus chloramphenicol (4 mg/ml)	9 ± 3
Epinephrine (9 μg/ml)	27 ± 6
Phentolamine (0.5 μg/ml) plus epinephrine (9 μg/ml)	11 ± 3
Phentolamine (0.5 μg/ml) plus chloramphenicol (4 mg/ml) plus epinephrine (9 μg/ml)	3 ± 1

[a] Values are means of six observations ± *SE*.

was added after 0.5 hr. This dose of phentolamine was sufficient to block the maximum epinephrine-induced relaxation, indicating the total block of α-adrenergic receptors. Chloramphenicol, 2 mg/ml, added with both blocker (2.5 μg/ml) and challenger (0.267 μg/ml) present, produced the same degree of relaxation as the control chloramphenicol response.

Effect of Chloramphenicol on Isolated Rabbit Jejunum—The minimum concentration of chloramphenicol to inhibit the frequency and amplitude of spontaneously occurring rhythmic pendular contraction was 250 μg/ml of bath. Incremental increases of chloramphenicol concentration from 250 μg/ml to 2 mg/ml induced a dose-dependent reduction in the frequency of spontaneous contractions. At concentrations above 2 mg/ml, the jejunum became paralyzed; the normal control rhythm could be restored after washing (Table V).

When the jejunum was stimulated with 0.01 μg of acetylcholine/ml, the frequency of pendular contractions, 10/min, did not increase. However, there was a marked increase in the tone, as evidenced by the uplift of the baseline in the record of jejunal contraction. The addition of 1 mg of chloramphenicol/ml in the presence of the same amount of acetylcholine led to a reduction of the frequency of contraction to 4.5/min. This rate was further reduced to 1.25/min when the concentration of chloramphenicol was increased to 2 mg/ml. When the dose of acetylcholine was increased to 0.02 μg/ml, the jejunal contraction could be reduced considerably by 1–2 mg of chloramphenicol/ml (Table V).

When barium chloride was added as a nonspecific stimulant in concentrations of 25 μg/ml, the frequency of jejunal contractions did not change significantly. Addition of chloramphenicol, 0.5–1.0 mg/ml, considerably reduced the frequency of jejunal contraction. The reduction was greater when the concentration of chloramphenicol was 1 mg/ml. The reduction could be reversed by the removal of chloramphenicol by washing (Table V).

Table V—Effect of Chloramphenicol on the Pendular Contractions of the Isolated Rabbit Jejunum

Drugs Used per Milliliter of Bath	Pendular Contractions per Minute[a]
Control	11 ± 1
Chloramphenicol (0.25 mg)	10 ± 1
Chloramphenicol (0.5 mg)	9 ± 1
Chloramphenicol (1.0 mg)	6 ± 1
Chloramphenicol (2.0 mg)	3 ± 1
Acetylcholine (0.01 μg)	10 ± 0.5
plus chloramphenicol (1 mg)	4.5 ± 1
plus chloramphenicol (2 mg)	1.25 ± 0.25
Acetylcholine (0.02 μg)	10 ± 0.3
plus chloramphenicol (1 mg)	3.5 ± 0.4
plus chloramphenicol (2 mg)	1.18 ± 0.2
Barium chloride (25 μg)	9.5 ± 1
plus chloramphenicol (0.5 mg)	5.5 ± 1
plus chloramphenicol (1 mg)	3.25 ± 0.4

[a] Values are means of six observations ± *SE*.

Table VI—Effect of Chloramphenicol on the Height of Contraction of Guinea Pig Trachea in the Presence of Agonist and Blocking Agents

Drugs Used per Milliliter of Bath	Height of Contraction[a], mm
Acetylcholine (4 μg)	15.5 ± 0.9
plus chloramphenicol (0.5 mg)	10.1 ± 0.7
plus chloramphenicol (1 mg)	8.0 ± 0.3
plus chloramphenicol (2 mg)	5.0 ± 0.8
plus chloramphenicol (4 mg)	2.0 ± 0.4
Histamine (7 μg)	22 ± 3.4
plus chloramphenicol (1 mg)	18 ± 2.3
plus chloramphenicol (2 mg)	11.5 ± 1.4
plus chloramphenicol (4 mg)	4.6 ± 1.0
Diphenhydramine (2.5 mg) plus histamine (7 μg)	5.3 ± 0.4
Diphenhydramine (2.5 mg) plus histamine (7 μg) plus chloramphenicol (1 mg)	4.0 ± 1.1
Propranolol (2.5 μg) plus histamine (7 μg)	20 ± 2.2
Propranolol (2.5 μg) plus histamine (7 μg) plus chloramphenicol (2 mg)	10 ± 1.1

[a] Values are means of six observations ± *SE*.

Effect of Chloramphenicol on Isolated Guinea Pig Trachea—The minimum amount of chloramphenicol required to produce relaxation in the guinea pig trachea was 0.5 mg/ml of bath. An increased concentration of chloramphenicol produced a dose-dependent reduction in the height of contraction. The addition of 4 μg of acetylcholine or of 7 μg of histamine/ml produced a sustained contraction of the trachea, which could be antagonized minimally by 0.5 mg of chloramphenicol in the case of acetylcholine and by 1.0 mg of chloramphenicol in the case of histamine and maximally in each instance by 4 mg/ml.

When chloramphenicol was added before the addition of histamine in the bath, the histamine response diminished. The inhibition was 19, 43, and 79% for 1, 2, and 4 mg of chloramphenicol/ml, respectively. The behavior of chloramphenicol was much like that of an antihistaminic drug. Similarly, chloramphenicol was added to the bath before the addition of acetylcholine and the acetylcholine response diminished. The inhibition was 38, 47, 68, and 87% for 0.5, 1, 2, and 4 mg of chloramphenicol/ml, respectively (Table VI).

Diphenhydramine hydrochloride, 2.5 μg/ml, inhibited the histamine response by approximately 76%. The addition of chloramphenicol in the bath, 1 mg/ml, further reduced the height of contraction; the total inhibition was 82%. Propranolol hydrochloride, 2.5 μg/ml, also reduced the histamine response (10%), but the addition of chloramphenicol to propranolol reduced the histamine response to a great extent (55%) (Table VI).

Effect of Chloramphenicol on Release of Calcium in Bath Fluid—In experiments with rabbit jejunum, calcium- and magnesium-free Tyrode solution was used to exclude the possibility of effects of chloramphenicol on these ions. The isoosmolarity of the fluid was maintained by the addition of extra sodium chloride. Control experiments with Tyrode solution containing the usual concentrations of calcium and magnesium were also undertaken.

After chloramphenicol was added to the bath to study its effect on the contraction of jejunum, no change in the concentration of calcium ion in the bath fluid could be observed. In experiments with rabbit aortic strips, calcium and magnesium concentrations in the bath fluid were maintained as usual. The presence of chloramphenicol did not influence the concentration of calcium in the bath, indicating no change in the availability of calcium to the tissues.

DISCUSSION

Chloramphenicol not only inhibited the autogenous contractile mechanism but was also active when the contractile drive was magnified by the use of agonists. The degree of muscle relaxation was related to the concentration of chloramphenicol in the bath. The action could be reversed by removing chloramphenicol by washing the organ preparation.

The force of contraction of the guinea pig trachea, enhanced by either histamine or acetylcholine, was reduced by the addition of chloramphenicol in the bath. Chloramphenicol, when added to the bath prior to the addition of histamine or acetylcholine, partially counteracted the action of the latter drugs. Chloramphenicol thus behaved as an antihistaminic or as an acetylcholine blocker. Furthermore, chloramphenicol reduced the force of contraction in the presence of propranolol (β-block) and diphenhydramine (histamine block).

In experiments with the toad heart, 0.2 μg of propranolol/ml blocked completely the maximal β-receptor-induced response. In experiments with tracheal chains, 2.5 μg of propranolol hydrochloride/ml was used, 12.5 times the dose used in toad heart experiments. Furthermore, Black and Stephenson (8) showed that the effect of epinephrine on the guinea pig tracheal chain could be antagonized by 0.1 μg/ml or less of pronethalol, a drug of one-tenth the potency compared to propranolol with regard to its β-adrenergic blocking property (9). It was, therefore, quite likely that the β-block was complete with 2.5 μg of propranolol hydrochloride/ml.

Diphenhydramine hydrochloride, 2.5 μg/ml, produced a relaxation of about 76% of the control histamine response. A further increase in the dose produced variable results. If a total block of histamine response by diphenhydramine hydrochloride was attained, no further relaxant effect by chloramphenicol could be demonstrated. Complete histaminergic block with diphenhydramine hydrochloride was not attempted. These findings indicated that chloramphenicol possibly did not act through either the adrenergic β-receptor or histaminergic receptor present in the tracheal tissue.

Similar action by chloramphenicol was observed in experiments with the toad heart. Chloramphenicol depressed the force and rate of contraction even in the presence of atropine sulfate and propranolol hydrochloride. Acetylcholine chloride, 0.04 μg, completely stopped the toad heart beat. Atropine sulfate, 0.25 μg/ml, completely blocked the effect of acetylcholine in all experiments. This dose of atropine sulfate exerted a maximum anticholinergic effect. Therefore, chloramphenicol-induced relaxation was not achieved through β-receptor blockade or cholinergic stimulation.

By gradually increasing the concentration of physostigmine in the perfusion fluid of control experiments with the toad heart, it was observed that physostigmine, 4.7 μg/ml of perfusion fluid, maximally potentiated the acetylcholine response, suggesting the achievement of maximal anticholinesterase activity. The maximal cholinesterase-inhibiting dose of physostigmine (4.7 μg/ml) alone in the perfusion fluid could not significantly inhibit either the rate or amplitude of contraction as compared to the control values (Table III). Chloramphenicol, 3.4 mg, produced a rate inhibition of 14 beats/min and an amplitude inhibition of 57 mm with respect to the control values. The response to chloramphenicol in the presence of physostigmine at the dose level of 4.7 μg/ml, which inhibited the cholinesterase enzyme completely, remained unaltered; *i.e.*, the difference between control chloramphenicol and chloramphenicol with physostigmine values was not statistically significant (Table III).

It, therefore, seems that the acetylcholinesterase inhibition by physostigmine could not provide further effective concentration of acetylcholine to achieve an enhanced cholinergic effect. If chloramphenicol promoted the release of acetylcholine, there would have been a significant difference between the chloramphenicol and chloramphenicol with physostigmine values. This result indicated the absence of involvement of acetylcholine in the characteristic chloramphenicol-induced muscle relaxation.

In the rabbit aortic strip preparation, chloramphenicol produced muscle relaxation even after a partial α-receptor block with phentolamine. But in the rabbit ileum preparation, chloramphenicol produced muscle relaxation even after a complete α-receptor block with phentolamine. This finding indicated that relaxation was not mediated through the α-receptor mechanism.

In experiments with the rabbit jejunum, which has a weak autogenous contractile drive, chloramphenicol reduced both the force and frequency of the spontaneously occurring pendular contraction. The minimum amount of chloramphenicol needed to demonstrate these effects was 250 μg/ml. The same amount of chloramphenicol was also needed to demonstrate its effects on the toad heart, which has a stronger spontaneous autogenous drive. To produce about 65% inhibition in the frequency of contraction, however, the amount of chloramphenicol needed per milliliter of bath was 1.5 mg for rabbit jejunum (Table V) and 8 mg for toad heart (Table II). This difference seemed to be logical. The toad heart has a greater autogenous contractile drive than the jejunum. To achieve the same percent inhibition, more chloramphenicol would seem necessary in the toad heart than in the jejunum.

The contractile force and frequency of contraction of the jejunum preparation, increased by pretreatment with acetylcholine, could be reduced by the addition of chloramphenicol in the bath (Table V), indicating that chloramphenicol could interfere with either drug-induced or spontaneously occurring contraction. The ability of chloramphenicol to antagonize the direct stimulation of the jejunum by barium chloride (Table V) indicated that chloramphenicol was interfering with the energy-generating mechanism (3).

Experiments with guinea pig ileum, rat fundal strip, rat colon, rat estrous uterus, and rat vas deferens, using various agonists, clearly demonstrated that chloramphenicol inhibited smooth muscles nonspecifically, acting as a nonspecific spasmolytic substance. From these observations, it may be concluded that chloramphenicol induces relaxation of smooth muscles by a direct action.

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ACKNOWLEDGMENTS AND ADDRESSES

Received December 9, 1974, from the *Research and Development Division, Dey's Medical Stores (Manufacturing) Private Limited, Calcutta-19, India.*

Accepted for publication July 22, 1975.

The authors thank Sri B. N. Dey, Dey's Medical Stores (Manufacturing) Limited, for the facilities provided.

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