Smooth Muscle Relaxant Properties and Vasomotor Actions of 2-Amino-1-*p*-nitrophenylpropane-1,3-diol

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Abstract □ Experiments with the guinea pig ileum, trachea, and vas deferens, the rat fundal strip, the rabbit jejunum and aortic strip, and the toad heart indicated that 2-amino-1-*p*-nitrophenylpropane-1,3-diol, the hydrolytic product of chloramphenicol, inhibited smooth muscles. Its action was direct and not through any mediators. After intravenous administration, the compound produced vasodepression followed by an overshooting rise of blood pressure. Vasodepression was not mediated by adrenergic, cholinergic, or histaminergic mechanisms. Hypertension was a sympathomimetic effect. Muscle relaxant and cardiovascular effects of the compound were similar to those of chloramphenicol, although it had no antibacterial effect.

Keyphrases □ 2-Amino-1-*p*-nitrophenylpropane-1,3-diol—evaluated for smooth muscle relaxant and vasomotor activities, various guinea pig, rat, rabbit, and toad muscles □ Chloramphenicol and hydrolytic product—evaluated for smooth muscle relaxant and vasomotor activities, various guinea pig, rat, rabbit, and toad muscles □ Relaxants, smooth muscle—chloramphenicol and its hydrolytic product evaluated, various guinea pig, rat, rabbit, and toad muscles □ Vasomotor activity—chloramphenicol and its hydrolytic product evaluated, various guinea pig, rat, rabbit, and toad muscles

In an earlier study (1), chloramphenicol inhibited smooth muscles, decreasing both the height and frequency of spontaneous contractions; the induced relaxation was not mediated through adrenergic, cholinergic, or histaminergic mechanisms. Chloramphenicol is hydrolyzed to 2-amino-1-*p*-nitrophenylpropane-1,3-diol (I), which has no antibacterial properties (2). Therefore, it was of interest to determine if I has muscle relaxant properties like chloramphenicol (II) to associate or dissociate the antibacterial and muscle relaxant properties of II. Cardiovascular effects of these two compounds also were investigated.

EXPERIMENTAL

The experimental procedures with smooth muscles of various animal species were the same as described earlier for II (1). Compound I was used in place of II.

Adult cats of either sex, 2.5–4 kg, were anesthetized with phenobarbital sodium, 150 mg/kg im. Standard pharmacological methods (3) were used for the cat spinal preparation and blood pressure recording.

RESULTS

Effect of I—*On Isolated Guinea Pig Ileum*—The agonist was acetylcholine. A significant alteration in the height of contraction of the guinea pig ileum was observed with a relatively higher dose of I, 80 μg/ml of bath. Incremental increases in concentrations from 80 to 1280 μg/ml of bath progressively inhibited the height of contraction (Table I).

On Isolated Rat Fundal Strip, Isolated Guinea Pig Trachea, Isolated Guinea Pig Vas Deferens, and Isolated Rabbit Aortic Strip—The agonists used were 5-hydroxytryptamine, histamine, epinephrine, and epinephrine for the fundal strip, trachea, vas deferens, and aortic strip, respectively. The height of contraction (relaxation in the guinea pig trachea) was significantly altered at a relatively high concentration of I, 250 μg/ml of bath. Incremental increases in I concentration from 250 μg to 2 mg/ml of bath progressively reduced the height of contraction (Table I).

On Perfused Toad Heart—Reductions in the rate and height of contraction of the spontaneously beating toad heart could be observed when the minimum dose of I was 250 μg. Incremental increases in the concentration of I from 250 μg to 2 mg progressively reduced the amplitude of contraction, but the frequency of the beat changed significantly only when the I dose was 2 mg, which produced a state of heart contracture (Table II). Although the action of the compound was similar to that of chloramphenicol, a much higher dose of the latter was needed to change significantly the frequency of the heart beat and to inhibit contraction totally (1).

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

Tissue Preparation	Agonist, μg/ml of Bath	Compound I, μg/ml of Bath	Inhibition Compared to Agonist-Induced Contraction[a], %
Guinea pig ileum	Acetylcholine, 0.66	80	23 ± 4
		160	40 ± 4
		320	52 ± 15
		640	66 ± 15
		1280	88 ± 3
Rat fundus strip	5-Hydroxytryptamine, 0.23	250	20 ± 2
		500	32 ± 3
		1000	63 ± 7
		2000	79 ± 1
Guinea pig trachea	Histamine, 6.0	250	11 ± 2
		500	29 ± 1
		1000	54 ± 6
		2000	80 ± 4
Guinea pig vas deferens	Epinephrine, 1.4	250	6 ± 0.5
		500	19 ± 1
		1000	45 ± 1
		2000	92 ± 3
Rabbit aortic strip	Epinephrine, 15	250	20 ± 2
		500	51 ± 5
		1000	83 ± 6
		2000	90 ± 3

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

Epinephrine and histamine were used, and Compound I reduced the force and rate of contraction of the heart stimulated by these agonists. The protocol and pattern of experiments were similar to those for chloramphenicol (1).

To ascertain that the action of I was direct and not mediated through inhibition or release of mediators, the effects of several blocking agents were studied. The experiments were similar to those described for chloramphenicol (1). The action of I was direct and not influenced by mediators (Table III).

On Isolated Rabbit Ileum—Epinephrine, 0.133 μg/ml of bath, produced maximal relaxation. An increase in the concentration of epinephrine to 0.267 μg/ml of bath diminished the relaxation response. Phentolamine hydrochloride, 2.5 μg/ml, was added to the bath. After 0.5 hr, epinephrine, 0.133 μg/ml of bath, was added. The dose of phentolamine was sufficient to block the maximum epinephrine-induced relaxation, indicating the total block of adrenergic α-receptors. Compound

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

Dose of I, mg	Inhibition[a], % Frequency/min	Inhibition[a], % Amplitude, mm
0.25	16 ± 3	20 ± 3
0.50	18 ± 6	30 ± 4
1.00	22 ± 4	68 ± 2
2.00	78 ± 4	100 (total)

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

Table III—Effect of I on the Perfused Toad Heart with Various Agonists and Blocking Agents [a]

Control	Effect of I	Agonist or Blocker Used	Effect of Agonist or Blocker	Combined Effect of Agonist or Blocker plus I
		Heart Beats per Minute		
44 ± 2	33 + 2 (3 mg)	Epinephrine (0.2 μg)	57 ± 1, $p < 0.005$	40 ± 4
41 ± 0.5	20 + 5 (3 mg)	Histamine (10 μg)	53 ± 1, $p < 0.005$	13 ± 4
55 ± 2	22 ± 5 (3 mg)	Propranolol (0.2 μg/ml)	50 ± 3	17 ± 6
69 ± 2	35 + 2 (4 mg)	Atropine (0.25 μg/ml)	68 ± 4	31 ± 3
44 ± 1	29 ± 1 (4 mg)	Physostigmine (5 μg/ml)	39 ± 1	23 ± 4
		Height of Contraction, mm		
24 ± 0.5	Complete inhibition (3 mg)	Epinephrine (0.2 μg)	69 ± 1, $p < 0.005$	Complete inhibition
25 ± 1	Complete inhibition (3 mg)	Histamine (10 μg)	32 ± 5	Complete inhibition
25 ± 2	Complete inhibition (3 mg)	Propranolol (0.2 μg/ml)	20 ± 3	Complete inhibition
64 ± 3	Complete inhibition (4 mg)	Atropine (0.25 μg/ml)	64 ± 7	Complete inhibition
29 ± 1	Complete inhibition (4 mg)	Physostigmine (5 μg/ml)	25 ± 2	Complete inhibition

[a] Values are means of seven observations ± *SE*. Figures in parentheses are the doses of the drug. Total doses of I and agonists are per milliliter of perfused fluid. Differences in the values in columns 1 and 4 and in columns 2 and 5 are not statistically significant except where *p* values are given.

I, 2 mg/ml of bath, when added to the combined presence of blocker (phentolamine) and challenger (epinephrine), produced almost the same degree of relaxation as the control response of I.

On Isolated Rabbit Jejunum—The dose, pattern, and protocol of experiments were similar to those used with II (1). The minimum concentration of I to produce alteration in the frequency and amplitude of spontaneously occurring rhythmic pendular contraction was 250 μg/ml of bath. Higher concentrations produced a dose-dependent reduction in the frequency of spontaneous contractions. At concentrations above 2 mg/ml of bath, the jejunum was paralyzed. The normal control rhythm, however, could be restored by washing (Table IV).

When the jejunum was stimulated with 0.01 μg of acetylcholine/ml of bath, the frequency of pendular contractions, 15/min, did not change. The addition of 1 mg of I/ml of bath in the presence of the same amount of acetylcholine reduced the frequency of contractions to four/min (Table IV). When barium chloride was added as a nonspecific stimulant, 25 μg/ml of bath, the frequency of jejunal contractions did not change significantly. Addition of I, 2 mg/ml, reduced the frequency of contractions from 12 to five/min. The reduction could be reversed by washing (Table IV).

On Isolated Guinea Pig Trachea—The protocol and pattern of experiments were similar to those used with II (1). Addition of 6 μg of histamine/ml of bath produced a sustained contraction of the trachea, which could be antagonized minimally by 0.25 mg of I/ml and maximally by 2 mg/ml. When I was added before histamine to the bath, the histamine response diminished. The reduction in the height of contraction was dose dependent (Table V).

Diphenhydramine hydrochloride, 2.5 μg/ml, inhibited the histamine response by approximately 76%. The addition of I in the bath, 1 mg/ml, further reduced the height of contraction; the total inhibition was 86%. Propranolol hydrochloride, 2.5 μg/ml, also reduced the histamine response by 10%, but the addition of I to propranolol reduced the histamine response to 82% (Table V).

On Calcium Release in Bath Fluid—Experiments were performed with the isolated rabbit jejunum and rabbit aortic strip as described previously for II (1). The presence of I did not influence the concentration of calcium in the bath, indicating no change in the availability of calcium in the tissues.

Cardiovascular Response to I and II—In experiments with cats, intravenous injections of both I and II, 2.5–50 mg/kg, progressively decreased blood pressure. The vasodepressor responses at higher dose levels were prompt and longer in duration (5–10 min). Repeated intravenous administration, 2.5 mg/kg, at 5-min intervals over 30 min, showed no evidence of tachyphylaxis or anaphylaxis; *i.e.*, the vascular response neither decreased nor increased. Regardless of the dose–response relationship, blood pressure did not promptly return to the preinjection control levels. The responses of I were similar to those of II.

Vasodepressive Response to I and II—*With Antihistaminic*—To determine if the vasodepressor response to I and II was due to the release of endogenous histamine, I and II were administered 30 min before and after producing histamine block in cats. An intravenous test dose of histamine acid phosphate, 0.5 μg/kg, was injected 20 min before and after the intravenous injection of antazoline methanesulfate, 4 mg/kg, to examine the efficiency of the histamine block. After a satisfactory, but varying, degree of histamine block was established in each animal, the intravenous injection of I and II, 20 mg/kg, produced a vasodepressor response in all animals that was identical in pattern and degree to the preblock control response (Table VI).

With β-Adrenergic Blockade—To determine if I and II produced the vasodepressor response through direct stimulation of β-adrenergic receptors, they were injected intravenously, 20 mg/kg, to cats before and after complete β-receptor block by propranolol hydrochloride, 0.3 mg/kg. To appraise the adequacy of the block, a challenging dose of isoproterenol sulfate, 0.01 mg/kg, was administered intravenously after the propranolol injection. No significant alteration in the vasodepressor responses of I and II was observed between the pre- and postblocked conditions (Table VI).

With Atropine—To determine if I and II produced the vasodepressor response by elaboration of mediator acetylcholine, they were administered before and after producing acetylcholine block in cats. An intravenous test dose of acetylcholine, 1 μg/kg, was administered 15 min before and 45 min after atropine sulfate, 1 mg/kg iv, to examine the efficacy of the block. The marked vasodepressor responses to acetylcholine were blocked adequately by atropine sulfate in all experiments. After the block was established, the intravenous injection of both I and II, 20 mg/kg, produced a vasodepressor response in all animals that was identical in pattern and degree to the preblock control response (Table VI).

With α-Adrenergic Blockade—Compounds I and II were injected

Table IV—Effect of I on Pendular Contractions of the Isolated Rabbit Jejunum

Drugs Used per Milliliter of Bath	Pendular Contractions per Minute [a]
Control	12 ± 0.44
I, 0.25 mg	11 ± 0.40
I, 0.50 mg	10 ± 0.40
I, 1.00 mg	7 ± 1.00
I, 2.00 mg	2 ± 0.50
Acetylcholine, 0.01 μg	15 ± 0.45
plus I, 1 mg	4 ± 1.00
Barium chloride, 25 μg	12 ± 0.45
plus I, 2 mg	5 ± 1.00

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

Table V—Effect of I on the Height of Contraction of Guinea Pig Trachea with Agonist and Blocking Agents

Drugs Used per Milliliter of Bath	Height of Contraction [a], mm
Histamine, 6 μg	13.0 ± 0.47
plus I, 0.25 mg	11.5 ± 0.47
plus I, 0.50 mg	9.0 ± 0.57
plus I, 1 mg	6.0 ± 1.00
plus I, 2 mg	2.5 ± 0.70
Histamine, 7 μg	22.0 ± 3.40
Diphenhydramine, 2.5 μg, plus histamine, 7 μg	5.3 ± 0.40
Diphenhydramine, 2.5 μg, plus histamine, 7 μg, plus I, 1 mg	3.0 ± 0.57
Propranolol, 2.5 μg, plus histamine, 7 μg	20.0 ± 2.20
Propranolol, 2.5 μg, plus histamine, 7 μg, plus I, 2 mg	4.0 ± 1.00

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

Table VI—Effect of Pretreatment with Blocking Agents on the Vasodepressor Action of I and II in Anesthetized Cats [a]

Reduction with I or II, %	Blocker Used	Reduction in Blood Pressure Using Blockers followed by I or II, %
	II, 20 mg/kg	
56 ± 6	Antazoline methanesulfate, 4 mg/kg	56 ± 8
42 ± 5	Atropine sulfate, 1 mg/kg	41 ± 5
45 ± 5	Propranolol hydrochloride, 0.3 mg/kg	41 ± 5
43 ± 4	Phentolamine hydrochloride, 5 mg/kg	44 ± 4
38 ± 5	Physostigmine salicylate, 0.3 mg/kg	48 ± 3
	I, 20 mg/kg	
38 ± 6	Antazoline methanesulfate, 4 mg/kg	35 ± 4
47 ± 6	Atropine sulfate, 1 mg/kg	45 ± 5
33 ± 4	Propranolol hydrochloride, 0.3 mg/kg	39 ± 4
33 ± 6	Phentolamine hydrochloride, 5 mg/kg	43 ± 6
40 ± 13	Physostigmine salicylate, 0.3 mg/kg	49 ± 14

[a] Values are means of five observations ± *SE*. The mean control blood pressure was 90 ± 7 mm Hg.

intravenously, 20 mg/kg, to cats before and after complete α-receptor blockade with phentolamine hydrochloride, 5 mg/kg. The adequacy of the block was tested by intravenous injection of a challenging dose of epinephrine, 1 μg/kg, after the injection of phentolamine. The vasodepression observed was similar in all respects to the pretreated control response of I and II in all animals (Table VI).

With Physostigmine—To determine if the vasodepressor action of I and II was potentiated or prolonged by acetylcholinesterase inhibition, they were injected, 20 mg/kg, before and after the intravenous administration of physostigmine salicylate, 0.3 mg/kg. One hour after physostigmine injection, there was no significant change in the vasodepressor response to either I or II (Table VI). Atropine block of the muscarine effects of the elevated acetylcholine level induced by physostigmine also failed to modify the characteristic vasodepressor response to both I and II.

Effect of Spinal Transection or Vagotomy on Vasodepressor Action of I and II—Elimination of effects of higher centers by spinal transection or of vagal stimulation by bilateral vagotomy could not modify the vasodepressive effect of either I or II.

Table VII—Vasopressor Response of I and II in Anesthetized Cats

Drug Used	Rise in Blood Pressure[a], %
II, 20 mg/kg	39 ± 9
plus pretreatment for 1 hr with phentolamine, 5 mg/kg	21 ± 7
plus pretreatment for 1 hr with propranolol, 0.3 mg/kg	10 ± 5
I, 20 mg/kg	31 ± 5
plus pretreatment for 1 hr with phentolamine, 5 mg/kg	39 ± 4
plus pretreatment for 1 hr with propranolol, 0.3 mg/kg	0
Epinephrine, 1 μg/kg	27 ± 5
plus pretreatment for 1 hr with II	76 ± 7
Epinephrine, 2 μg/kg	44 ± 8
plus pretreatment for 1 hr with II	82 ± 9
Epinephrine, 4 μg/kg	50 ± 15
plus pretreatment for 1 hr with II	94 ± 12
plus pretreatment for 1 hr with I	93 ± 5

[a] Values are means of five observations ± *SE*. The mean control blood pressure was 88 ± 3 mm Hg.

Response to I and II with β-Adrenergic, Acetylcholinergic, or Histaminergic Blockades in Isolated Rabbit Aortic Strip Preparation—Both I and II produced significant dose-dependent relaxation in the aortic strip after a contraction was produced by the agonist epinephrine. After a satisfactory epinephrine-induced contraction for 4 min, either I or II, 4 mg/ml of bath, was added at the peak height of contraction. The muscle relaxation effect immediately ensued, continued for the next 6 min, and was not inhibited by propranolol (β-adrenergic blocker), atropine (cholinergic blocker), or antazoline (histaminergic blocker) in concentrations of 40 μg/ml of bath added to the bath 10–15 min prior to the addition of I or II.

Vasopressor Response of I and II in Cats—The vasodepression produced by I and II was followed by a compensatory overshooting rise of blood pressure much higher than the basal blood pressure. The response persisted after spinal transection. After α-receptor block with phentolamine, 5 mg/kg, for 1 hr, I and II behaved differently. The after rise of blood pressure was enhanced by I and did not change significantly with II. After β-receptor block with propranolol, 0.3 mg/kg for 20–30 min, the vasopressor effects of I and II were checked. Epinephrine injected intravenously in cats pretreated with I and II produced a potentiated vasopressor response in all animals in comparison with the control epinephrine-induced vasopressor response (Table VII). The response was present in spinal transected preparations also.

DISCUSSION

Experiments with I clearly indicated a close correlation of results obtained with II (1). Like II, I not only inhibited the autogenous contractile drive but also was active when the contractile mechanism was magnified by agonists. Compound I inhibited smooth muscles, nonspecifically acting as a nonspecific spasmolytic substance, and the muscle relaxation seemed due to a direct action like that of II.

The most significant cardiovascular response to both I and II was the persistent hypotensive action on rapid intravenous injection, which could not be inhibited by any of the mediator blocking agents (Table VI). Both I and II had relaxation effects on rabbit aortic strips not influenced by propranolol, atropine, and antazoline. Therefore, I and II possibly acted directly on vascular smooth muscle, leading to vasodepression in intact animals.

Vasodepressive action of I and II was followed by an overshooting rise of blood pressure, which could be prevented by β-receptor block. Epinephrine-induced vasopressor response was potentiated after intravenous administration of I and II (Table VII). These observations indicated that the rise in blood pressure after intravenous administration of I and II was possibly due to a sympathomimetic effect.

On hydrolysis, II loses dichloroacetic acid and I is formed, which has no bactericidal property (2). The smooth muscle relaxation properties of II seem to be due to the I moiety.

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