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Keyphrases

Carbonate ester hydrolysis
α-Chymotrypsin catalyzed hydrolysis
UV spectrophotmetry, continuous-analysis
Stoichiometry
pH-dependence of hydrolysis

Effect of Certain Drugs on Perfused Human Placenta VIII

Angiotensin-II Antagonists

By CHARLES O. WARD and RONALD F. GAUTIERI

The majority of the drugs tested as angiotensin antagonists in this investigation were from two pharmacologic classes: negative musculotropic agents (papaverine, sodium cobaltinitrite, isosorbide dinitrate, nitroglycerin, sodium nitrite, and dipyridamole) and agents which selectively block α-adrenergic receptors (dibenamine, phentolamine, tolazoline, and hydralazine). Of the direct smooth muscle depressants, sodium nitrite and dipyridamole were the most effective; dibenamine and phentolamine were the most potent adrenergic blocking agents employed. Atropine, cocaine, and cyproheptadine were also tested as antagonists of the pressor effect of angiotensin and exhibited only moderate effectiveness. Lidoflazine, a specific angiotensin antagonist, was the most potent compound tested in this study; the degree of antagonism exhibited by it, however, was only slightly greater than either dibenamine or phentolamine. The results indicate that the pressor effect of angiotensin in the perfused human placenta is primarily the result of a direct stimulation of vascular smooth muscle and secondarily to a stimulation of α-adrenergic receptors.

WHILE RESEARCH with angiotensin in the last two decades has dealt principally with its effects on innervated preparations, there still exists in the literature a paucity of information on the actions of this polypeptide in the more common nerve-free preparations, the perfused human placenta and umbilical cord. Eliasson and Astrom (1) could detect only moderate increases in perfusion pressure using angiotensin in the former preparation; others have reported little or no effect with angiotensin on either the perfused umbilical cord (2) or helical strips of both the human umbilical artery and vein (3). In contrast to these investigations, Gautieri and Mancini (4) recently reported an increase in perfusion pressure of the human placenta that ranged from 11 to 51 mm. Hg; this response to angiotensin was reduced but not completely blocked by either 3-or 6-mg. doses of phentolamine.

In addition to the effects of angiotensin on the above isolated preparations, many recent investigations have linked abnormally high blood levels of angiotensin and renin with the pathogenesis of the toxemias of pregnancy (5–8). The symptomology of clinical toxemia has been mimicked by the injection of renin-containing renal extracts into rats made hypertensive by the administration of desoxycorticosterone (DCA); renal damage similar to that of toxemia as well as convulsions, anasarca, oliguria or anuria, sodium retention, and increased blood urea nitrogen were reported (5). Also, an increased pressor response to angiotensin administration was found by Chesley (6) in women with pre-eclampsia. However, a decreased sensitivity to this autocoid was observed in normal pregnancy when this group was compared with

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nonpregnant controls. Dexter and Haynes (7) found higher than normal concentrations of renin in the blood of patients with eclampsia and pre-eclampsia and Landesman *et al.* (8) reported increased blood levels of angiotensinase in toxic women when compared with nontoxic, pregnant controls. When high doses of renin and angiotensin were administered to the pregnant rat, a drop in placental blood pressure was noted by Burlingame *et al.* (9). Injections of renin into the rat placenta itself produced consistent increases in perfusion pressures.

Thus, because angiotensin may be involved in the etiology of the toxemias of human pregnancy and because a previous study in this laboratory (4) has shown that angiotensin exerts its pressor action on perfused human placental vessels by both a stimulation of α-adrenergic receptors and also a direct stimulation of vascular smooth muscle, it was the purpose of this investigation to screen compounds that would selectively modify the mechanisms whereby angiotensin produces vasoconstriction in these vessels, in order not only to identify angiotensin antagonists, but also to more clearly elucidate its vasopressor action in this system.

MATERIALS AND METHODS

Full-term human placentas, obtained from the hospital 15 to 20 min. after normal delivery, were used throughout this investigation. Each was transported to the laboratory in a light-resistant glass container filled with approximately 1 L. of Tyrode's solution preheated to 38°.

The apparatus used and the procedures employed in the preparation and perfusion of the placentas, in recording and maintaining the perfusion pressure, and in measuring the inflow and outflow volumes of the perfusate have been described in previous papers (10, 11). In 38 successful placental preparations, each lasting 1–4 hr., a total of 106 studies was conducted. The results from 4 placentas were discarded because the response to a test dose of angiotensin was erratic.

Throughout this investigation the agonist, angiotensin, was administered before the various antagonists for the purpose of establishing a standard response to its vasoconstrictor effect; the antagonists were then administered 2–3 min. after the pressor response to angiotensin subsided. Subsequent responses to angiotensin, at 4–6-min. intervals after the pressor or depressor effect of the various antagonists subsided, were then compared to the initial standard. The mean pressure change at maximal antagonism of the pressor effect of angiotensin, as well as the mean onset of maximal antagonism in minutes, were used as bases for comparing the relative potencies of the various antagonists. The pressor dose of angiotensin used throughout this investigation was kept at 50.0 mcg. and was based on that used in a previous investigation (4). This dose of angiotensin produced a mean increase in perfusion pressure of 20.3 mm. Hg (range 10.6–34.0 and S. D. ±6.6).

The doses of the antagonists were determined by trial and error and were kept as low as possible, yet sufficient to exhibit angiotensin antagonism. However, in the case of the α-adrenergic blocking agents, their doses were based on those used to antagonize the constrictor effect of 40-mcg. doses of norepinephrine in a previous study with this preparation (11).

The following drugs were injected into the rubber tubing, prior to entering the pump, in a volume of distilled water not exceeding 5.0 ml., except where noted: angiotensin-II,[1] 0.01%; cyproheptadine hydrochloride,[2] 0.01%; papaverine hydrochloride USP, 1.0%; sodium cobaltinitrite, 0.4%; nitroglycerin USP, 0.1%; isosorbide dinitrate,[3] 0.4%; sodium nitrite, 0.4%; dipyridamole,[4] 0.5% in 1.0 ml. of an aqueous solution containing polyethylene glycol 600, 50 mg., and tartaric acid, 2 mg.; dibenamine hydrochloride,[5] 0.1% and 0.2% in 95% ethyl alcohol USP; phentolamine methanesulfonate,[6] 0.1%; tolazoline hydrochloride,[7] 0.1%; hydralazine hydrochloride,[8] 0.1%; lidoflazine,[9] 0.25% in 0.17 *M* acetic acid; atropine sulfate, 0.1%; cocaine hydrochloride USP, 1.0%. In addition, the appropriate amounts of 95% ethyl alcohol USP, 0.17 *M* acetic acid, and an aqueous solution containing 50 mg. of polyethylene glycol 600 and 2 mg. of tartaric acid per 1.0 ml. were injected to observe their effects on the placental vessels and the normal pressor response to angiotensin.

RESULTS

The following results, which are summarized in Table I, were obtained on the vasculature of full-term human placentas perfused at pressures between 70 and 120 mm. Hg. This range of perfusion pressures corresponds to inflow rates of 17 to 62 ml. of perfusion fluid (Tyrode's solution modified by the addition of 0.525% polyvinylpyrrolidone[10] per minute.

Cyproheptadine—In a dose of 100.0 mcg. cyproheptadine produced a mean pressure decrease of 3.32 mm. Hg (range 0.4 to 6.6) in the subsequent pressor responses to the standard 50.0-mcg. dose of angiotensin. In the five experiments performed, the maximum antagonism occurred an average of 24.4 min. (range 16.0 to 30.0) after the administration of the antagonist. The 200.0-mcg. dose of cyproheptadine produced a mean decrease of 3.6 mm. Hg (range 2.2 to 7.0) in perfusion pressure at maximum effectiveness. The onset of maximum antagonism

[1] Hypertensin, valyl-5-angiotensin-II amide, lot No. B-5578, supplied through the courtesy of Ciba Pharmaceutical Co., Summit, N.J.
[2] Periactin, supplied through the courtesy of Merck Sharp and Dohme Laboratories, West Point, Pa.
[3] Isordil, supplied through the courtesy of Ives-Cameron Laboratories, New York, N.Y.
[4] Persantin, supplied through the courtesy of Geigy Chemical Corp., Ardsley, N.Y.
[5] Supplied through the courtesy of the Givaudan Corp., Delawanna, N.J.
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[8] Apresoline, supplied through the courtesy of Ciba Pharmaceutical Co., Summit, N.J.
[9] Supplied through the courtesy of McNeil Laboratories, Fort Washington, Pa.
[10] Plasdone C.

TABLE I—COMPARISON OF ANGIOTENSIN ANTAGONISM BY SEVERAL COMPOUNDS IN PERFUSED HUMAN PLACENTAL VESSELS

Drug	Dose[a]	No. of Exp.	Av. Max. Antagonism, min.	Mean Pressure Decrease, mm. Hg	S.E.M.[b]	P Value[a]
Cyproheptadine	100.0 mcg.	5	24.4	3.32	±1.10	0.025
Cyproheptadine	200.0 mcg.	5	18.4	3.60	±1.31	0.050
Papaverine	5.0 mg.	5	19.0	2.88	±1.18	0.050
Papaverine	10.0 mg.	5	8.6	3.28	±0.74	0.010
Sodium cobaltinitrite	2.0 mg.	5	20.6	2.00	±0.53	0.010
Isosorbide dinitrate	2.0 mg.	5	25.6	1.36	±0.50	0.050
Nitroglycerin	2.0 mg.	5	18.4	3.12	±1.08	0.025
Sodium Nitrite	2.0 mg.	6	19.5	6.66	±2.11	0.025
Dipyridamole	5.0 mg.	5	14.4	5.16	±0.76	0.005
Dibenamine	2.0 mg.	5	16.2	2.08	±1.57	0.150[c]
Dibenamine	4.0 mg.	5	20.2	7.72	±1.29	0.005
Phentolamine	3.0 mg.	5	14.1	7.74	±2.32	0.025
Tolazoline	5.0 mg.	5	15.4	4.90	±1.56	0.025
Hydralazine	4.0 mg.	5	17.6	2.24	±0.43	0.005
Atropine	1.0 mg.	5	17.4	2.50	±0.50	0.005
Cocaine	2.0 mg.	6	13.3	1.73	±1.36	0.150[c]
Lidoflazine	5.0 mg.	5	17.4	8.86	±3.33	0.050

[a] P value calculated from "paired t test" (17). [b] S.E.M. = standard error of the mean. [c] P value >0.050 not significant.

occurred at about 18.4 min. (range 4.0 to 32.0) after 200.0 mcg. of cyproheptadine had been administered. In one of the five preparations the 200.0-mcg. dose of the antagonist resulted in a mild increase (0.4 mm. Hg) in the pressor response to subsequent doses of angiotensin. In 7 of the 10 experiments performed, using both doses of cyproheptadine, the antagonist itself produced an increase in perfusion pressure ranging from 1.0 to 9.2 mm. Hg; in the other three experiments no pressure change was noted immediately following the administration of the antagonist.

Papaverine—In a total of 5 experiments, a 5.0-mg. dose of papaverine produced an average decrease in the pressor response to angiotensin of 2.88 mm. Hg (range 2.8 to 6.2). In one instance a 1.2 mm. Hg increase in perfusion pressure, rather than a decrease, was noted when angiotensin was given following papaverine administration. The maximum decrease in the pressor response to subsequent doses of angiotensin occurred an average of 19.0 (range 7.0 to 52.0) min. after administration of the antagonist. In two cases the antagonist produced a decrease in perfusion pressure itself; an increase was noted immediately following the administration of the antagonist in one experiment; and no change was produced by papaverine itself in the remaining two experiments. In a dose of 10.0 mg. papaverine always produced a decrease in pressure by itself of 2.0 to 6.2 mm. Hg and the mean pressure decrease at maximum angiotensin antagonism was 3.28 mm. Hg (range 1.4 to 5.4) in the five experiments performed at this dosage. The greatest degree of antagonism occurred an average of 8.6 min. (range 5.0 to 14.0) after the higher dose of papaverine had been administered.

Sodium Cobaltinitrite—Sodium cobaltinitrite, in a dose of 2.0 mg., produced a mean decrease in the pressor response to subsequent doses of angiotensin of 2.0 mm. Hg (range 0.6 to 3.6) at maximum effectiveness. In the five experiments performed the maximum degree of angiotensin antagonism occurred an average of 20.6 min. (range 16.0 to 37.0) after administration of the antagonist. In all of the experiments performed, sodium cobaltinitrite produced a decrease in perfusion pressure itself of 0.8 to 6.6 mm. Hg.

Isosorbide Dinitrate—In a dose of 2.0 mg. isosorbide dinitrate reduced the pressor response to 50.0 mcg. of angiotensin an average of 1.36 mm. Hg (range 1.6 to 2.2) at maximal antagonism. In one of the five experiments performed the pressor response to angiotensin was increased 0.6 mm. Hg when compared to the original test dose of the agonist. An average of 25.6 min. (range 15.0 to 44.0) elapsed before the maximum effect of isosorbide dinitrate was noted; each administration of the antagonist was followed by a decrease in the perfusion pressure that ranged from 1.4 to 5.2 mm. Hg.

Nitroglycerin—In a total of five experiments 2.0 mg. of nitroglycerin produced an average decrease in the pressor response to subsequent angiotensin administration of 3.12 mm. Hg (range 2.4 to 5.0). The maximal efficacy of the antagonist was noted an average of 18.4 min. (range 14.0 to 25.0) after administration. In four instances the antagonist itself produced a decrease in perfusion pressure ranging from 1.4 to 14.4 mm. Hg.

Sodium Nitrite—Sodium nitrite, in a dose of 2.0 mg., produced a mean decrease in perfusion pressure of 6.66 mm. Hg (range 2.2 to 13.7) at maximum angiotensin antagonism. In one case a 1.6 mm. Hg increase in perfusion pressure was noted following angiotensin administration, when compared to the test dose of the agonist, after sodium nitrite had been given. In the six experiments performed the maximal antagonism of angiotensin occurred an average of 19.5 min. (range 11.0 to 38.0) after administration of the antagonist. In each case sodium nitrite itself produced a decrease in perfusion pressure of 1.0 to 5.2 mm. Hg.

Dipyridamole—In a total of five experiments 5.0 mg. of dipyridamole produced an average decrease in the pressor response to subsequent angiotensin administration of 5.16 mm. Hg (range 3.5 to 7.5). The maximum effect of the antagonist was noted an average of 14.4 min. (range 8.0 to 19.0) after administration. In each instance the antagonist itself produced a decrease in perfusion pressure of 2.0 to 5.2 mm. Hg immediately following its administra-

tion. A decrease in perfusion pressure (1.0 to 3.0 mm. Hg) was also noted when 1.0 ml. of an aqueous solution containing 50.0 mg. of polyethylene glycol 600 and 2.0 mg. of tartaric acid (equivalent to the vehicle for the 5.0-mg. dose of dipyridamole) was tested in four separate experiments.

Dibenamine—In a dose of 2.0 mg. dibenamine produced a mean pressure decrease of 2.08 mm. Hg (range 0.6 to 7.6) in the pressor response to the standard 50.0-mcg. dose of angiotensin. In the five experiments performed, the greatest decrease in the pressor response to angiotensin occurred an average of 16.2 min. (range 12.0 to 24.0) after the 2.0-mg dose of dibenamine had been given. In five separate experiments of 4.0-mg. dose of dibenamine produced a mean decrease of 7.72 mm. Hg (range 3.8 to 11.2 in the pressor response to subsequent doses of angiotensin at maximum effectiveness. The onset of maximal antagonism occurred at about 20.2 min. (range 11.0 to 28.0) after dibenamine had been given. The administration of 2.0 mg. of dibenamine produced an increase of 1.8 mm. Hg in the pressor response to subsequent doses of angiotensin in one preparation. In 9 of the 10 experiments performed, using both the 2.0- and 4.0-mg. doses of dibenamine, the antagonist itself produced an increase in perfusion pressure ranging from 1.6 to 27.0 mm. Hg. The administration of 1.0 ml. of 95% ethyl alcohol, equal to the amount used to dissolve the 2.0-mg. dose of dibenamine, resulted in a comparable increase in perfusion pressure (2.6 to 2.40 mm. Hg) in four separate experiments. Also, when 4.0 ml. of 95% ethyl alcohol, equivalent to the amount used to dissolve the 4.0-mg. dose of the antagonist, were tested in four experiments, an increase in perfusion pressure of 14.0 to 26.0 mm. Hg was observed.

Phentolamine—In a total of five experiments, a 3.0-mg. dose of phentolamine produced an average decrease in the pressor response to angiotensin of 7.74 mm. Hg (range 4.0 to 16.7). The maximum decrease in the pressor response to subsequent doses of angiotensin occurred an average of 14.1 min. (range 7.5 to 18.5) after the administration of the antagonist. In three cases the antagonist itself produced an increase in perfusion pressure ranging from 1.2 to 2.2 mm. Hg.

Tolazoline—Tolazoline, in a dose of 5.0 mg., produced a mean decrease in the pressor response to subsequent doses of angiotensin of 4.90 mm. Hg (range 4.0 to 8.8) at maximal antagonism. In one experiment tolazoline had no effect on the pressor response to angiotensin. In the five experiments performed the maximum degree of angiotensin antagonism occurred an average of 15.4 min. (range 11.0 to 23.0) after administration of tolazoline. In four of the experiments tolazoline itself produced an increase in perfusion pressure of 1.8 to 4.4 mm. Hg; in the one remaining instance a decrease in perfusion pressure (2.6 mm. Hg) was noted immediately after tolazoline had been given.

Hydralazine—In a dose of 4.0 mg. hydralazine reduced the pressor response to a standard 50.0-mcg. dose of angiotensin an average of 2.24 mm. Hg (range 1.0 to 4.4) at maximal antagonism. An average of 17.6 min. elapsed (range 13.0 to 23.0) before maximum the effect of hydralazine was noted. In each of the five experiments performed hydralazine administration was followed by an increase in perfusion pressure of 0.14 to 2.6 mm. Hg.

Atropine—Atropine, in a dose of 1.0 mg., produced a mean decrease in perfusion pressure of 2.50 mm. Hg (range 1.6 to 4.4) at maximum angiotensin antagonism. In the five experiments performed, the greatest decrease in the pressor response to angiotensin occurred an average of 17.4 min. (range 7.5 to 26.0) after the antagonist was administered. Atropine, itself, had no effect on perfusion pressure in any of the placentas studied.

Cocaine—In a dose of 2.0 mg. cocaine reduced the pressor response to a standard 50.0-mcg. dose of angiotensin an average of 1.73 mm. Hg (range 2.0 to 6.4) at maximal antagonism. An average of 13.33 min. elapsed (range 6.0 to 33.0) before the maximum effect of cocaine was noted. Cocaine itself had no effect on perfusion pressure in three experiments; in one case a decrease in perfusion pressure was noted; in the remaining two instances an increase in perfusion pressure was noted immediately after the antagonist was given. In only four of the six experiments performed did cocaine show an antagonism of the pressor effect of angiotensin; in the other two placentas increases in the pressor response to angiotensin (1.4 and 2.6 mm. Hg) were observed.

Lidoflazine—In a total of five experiments, 5.0 mg. of lidoflazine produced an average decrease in the pressor response to angiotensin of 8.86 mm. Hg (range 2.6 to 21.4). The maximum effect of the antagonist occurred an average of 17.4 min. (range 11.0 to 28.0) after administration. In each instance the antagonist itself produced a decrease in perfusion pressure of 2.6 to 8.0 mm. Hg immediately following its administration. The administration of an amount of 0.17 *M* acetic acid (2.0 ml.) equal to that used to dissolve the 5.0-mg. dose of lidoflazine produced a decrease (1.6 to 4.0 mm. Hg) in the perfusion pressure itself in three separate experiments; in another case it had no effect on perfusion pressure alone.

Polyethylene Glycol 600 and Tartaric Acid—The administration of 1.0 ml. of an aqueous solution, containing 50.0 mg. of polyethylene glycol 600 and 2.0 mg. of tartaric acid, slightly decreased thd pressor response to subsequent doses of angiotensin (0.2 mm. Hg) in one experiment. In three other experiments, however, an increase in the pressor response to the agonist (0.6 to 4.0 mm. Hg) was observed after injection of this solution.

Ethyl Alcohol—The administration of both 1.0 and 4.0 ml. of 95% ethyl alcohol caused no perceptible effect on the pressor responses of the placenta to subsequent doses of angiotensin in eight separate experiments.

Acetic Acid—A dose of 2.0 ml. of 0.17 *M* acetic acid in three experiments caused a small decrease (0.4 to 0.8 mm. Hg) in the pressor response to subsequent doses of angiotensin; in one other experiment an increase (1.0 mm. Hg) in the pressor response to angiotensin was observed.

DISCUSSION

Because a previous investigation in this labora-

tory (4) had demonstrated that the vasoconstrictor effect of angiotensin in the perfused human placenta was primarily the result of a direct stimulation of vascular smooth muscle and only partially due to a stimulant effect on α-adrenergic receptors, the majority of the drugs tested as antagonists in this investigation were chosen from two pharmacologic classes: negative musculotropic agents (papaverine, sodium cobaltinitrite, isosorbide dinitrate, nitroglycerin, sodium nitrite, and dipyridamole) and compounds that selectively block α-adrenergic receptors (dibenamine, phentolamine, tolazoline, and hydralazine).

Among the direct-acting smooth muscle depressants, sodium nitrite and dipyridamole were the most potent angiotensin antagonists and exhibited a mean pressure decrease at maximum antagonism of 6.66 mm. Hg and 5.16 mm. Hg, respectively. Isosorbide dinitrate, whose depressant effect on the placental vasculature has been shown to be due to the nitrate moiety (12), proved to be the weakest antagonist in this class (mean pressure decrease of 1.36 mm. Hg). However, its onset of action was longest, averaging 25.6 min. Each of the drugs in this category produced a mild and transient decrease in perfusion pressure which always subsided before its antagonistic effect on the pressor response to angiotensin was noted.

When 1.0 ml. of the vehicle used to dissolve dipyridamole (an aqueous solution containing 50.0 mg. of polyethylene glycol 600 and 2.0 mg. of tartaric acid per 1.0 ml.) was tested as an angiotensin antagonist, a slight increase (0.6 to 4.0 mm. Hg) in the pressor response to subsequent doses of angiotensin was observed in three experiments and a small decrease (0.2 mm. Hg) was noted in another. Thus it is reasonable to assume that the antagonistic ability of dipyridamole observed in this study is due entirely to the chemical itself, and not to the solvent employed.

Since previous investigations in this laboratory have shown that angiotensin and serotonin share similar mechanisms in producing a pressor effect on the vasculature of the perfused human placenta (4), and because cyproheptadine was shown to be a potent serotonin antagonist when tested in this preparation (13), this compound was evaluated as an angiotensin antagonist in this investigation. Only moderate angiotensin antagonism was recorded when cyproheptadine was tested in this study (mean pressure decreases of 3.32 mm. Hg with the 100.0-mcg. dose and 3.60 mm. Hg decrease with 200.0 mcg.). Cyproheptadine administration was followed by an immediate rise in perfusion pressure (range 1.0 to 9.2 mm. Hg) in this investigation whereas Gokhale *et al.* (2) observed no effect with this compound on the perfusion pressure of the human umbilical artery; yet they recorded a significant decrease, as in this study, in perfusion pressure immediately after administration of papaverine and sodium nitrite. In view of the above results, the negative musculotropic action for cyproheptadine, suggested as an additional mechanism of action by Ward and Gautieri (13), does not appear to be involved with its ability to antagonize the pressor effect of angiotensin in this preparation.

Dibenamine (mean pressure decrease of 7.72 mm. Hg in a dose of 4.0 mg.) and phentolamine (mean pressure decrease of 7.74 mm. Hg) were the most effective α-adrenergic receptor blocking agents employed in this study. In our results, the mean difference between the pressor effect of angiotensin before and after the administration of the 2.0-mg. dose of dibenamine was not statistically significant when the 0.050 level of probability was chosen after computation of the paired t test (17). Our results with the 3.0-mg. dose of phentolamine agree remarkably well with those of Gautieri and Mancini (4), who used a similar dose of this agent as an angiotensin antagonist in this preparation; they found, however, that doubling the dose of phentolamine did not increase its antagonistic ability. Our results with hydralazine (mean pressure decrease of 2.24 mm. Hg), in antagonizing the pressor effect of angiotensin in this isolated organ preparation, differ from those of Page and McCubbin (14) and Gross and Turrian (15), who could detect no antagonism of the pressor effect of angiotensin in the intact dog. Meier *et al.* (16), in contrast, have reported that hydralazine, but not phentolamine, reduced the pressor response to angiotensin in the perfused vasculature of the rabbit hind leg.

Although it was observed that the immediate increase in perfusion pressure of the human placenta, after the administraton of dibenamine, was due to the 95% ethyl alcohol used as the solvent, no angiotensin antagonism could be attributed to this vehicle when appropriate amounts were tested in eight separate experiments.

The foregoing results, therefore, indicate that the negative musculotropic agents employed produced the most predictable and consistent results of all the antagonists utilized. We are, then, in agreement with Gautieri and Mancini (4) who postulated that the pressor effect of angiotensin in the perfused human placental vessels was due primarily to a direct stimulation of vascular smooth muscle and only secondarily to a stimulation of α-adrenergic receptors.

A portion of angiotensin's stimulant effect on the guinea pig ileum, *in vitro*, has been shown to be the result of acetylcholine release from the ganglion cells in the muscular wall of the gut (18). Because of the above as well as the observations that acetylcholine is present normally in the human placenta (19) and elicits a vasoconstrictor effect on these vessels which is blocked by atropine (1), atropine was tested as an antagonist in an attempt to discern whether a portion of the pressor response to angiotensin in these nerve-free vessels (20) was the result of acetylcholine release. In this investigation atropine was only a weak angiotensin antagonist and produced a mean pressure decrease of 2.50 mm. Hg at maximum efficacy. From the results it is not possible, at this time, to attribute the antagonistic ability of atropine observed in these experiments to an antagonism of acetylcholine possibly released in response to angiotensin administration. Also, the observation that atropine can produce vasodilatation by a direct effect on vascular smooth muscle (21) cannot be ignored as a mechanism of angiotensin antagonism.

Since Distler *et al.* (22) have shown that angiotensin administration may result in the release of norepinephrine, a constrictor of placental vessels

(1, 11), from helical strips of the aorta and carotid artery of the pig, cocaine was chosen for testing as an antagonist because it has been shown to block the release of norepinephrine from storage sites in the terminals of sympathetic nerves (23). Cocaine appeared to be the least effective antagonist studied in the dose employed (mean pressure decrease of 1.73 mm. Hg) and the results obtained were not statistically significant ($P > 0.050$).

Lidoflazine, a specific angiotensin antagonist, has been shown to block the noncompetitive, indirect component of angiotensin's stimulant action on the guinea pig ileum *in vitro* (24); it was chosen for this study because it was the only specific angiotensin antagonist known. In this investigation lidoflazine was the most potent agent employed, as evidenced by a mean pressure decrease at maximal efficacy of 8.86 mm. Hg. Its onset of action (17.4 min.), however, was not greatly different from the other compounds employed. In four separate experiments an appropriate amount of 0.17 *M* acetic acid was tested for angiotensin antagonism because this was the solvent for lidoflazine. Only a negligible degree of antagonism (mean pressure decrease of 0.4 to 0.8 mm. Hg) was noted, indicating that the response to lidoflazine was not the result of an action of its solvent.

The results of this investigation indicate that the pressor response to angiotensin in the vasculature of the perfused human placenta is due principally to a direct stimulation of the vascular smooth muscle and, to a lesser degree, a stimulation of α-adrenergic receptors present in these vessels (11). No release of either acetylcholine or norepinephrine by angiotensin, as a portion of its pressor action in these vessels, can be postulated from the results of this study. It seems clear, therefore, that this preparation may be used to screen compounds that have potential therapeutic efficacy in the toxemias of pregnancy and as an aid in discerning the mechanisms of these and other vasoactive substances endogenous to the human body.

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Keyphrases

Angiotensin II antagonists
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Pressor response