Effect of Certain Drugs on Perfused Human Placenta IX: Mode of Action of Angiotensin

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Abstract □ By the use of compounds that directly depress vascular smooth muscle, block α-adrenergic receptors, or block β-adrenergic receptors, it was shown that a triple mechanism is involved in the pressor action of angiotensin in isolated, perfused human placental blood vessels. Angiotensin directly stimulates vascular smooth muscle and α-adrenergic receptors while concomitantly stimulating β-adrenergic receptors, to offset the effect of the first two mechanisms. Thus, when the β-receptors are blocked, the pressor response to angiotensin is augmented.

Keyphrases □ Placenta, human—angiotensin perfusion □ Angiotensin—activity mechanism □ Perfusion pressure, placenta—angiotensin effect □ Drug effect—angiotensin activity

Recent investigations with angiotensin in the isolated, perfused human placenta (1, 2) have characterized the pressor response of this autocoid as both stimulation of vascular smooth muscle as well as stimulation of α-adrenergic receptors known to be present in this organ (3). Negative musculotropic agents, such as sodium nitrite, dipyridamole, papaverine, and sodium cobaltinitrite (1) and certain α-adrenergic blocking agents, such as dibenamine, phenoxybenzamine, phentolamine, tolazoline, and hydralazine (1, 4, 5) have been shown to antagonize effectively the pressor response to this polypeptide.

Vogin and Buckley (5) have shown that in anesthetized dogs prior administration of the β-adrenergic blocking agents, pronethalol and dichloroisoproterenol (DCI), augmented the vascular response to angiotensin.

Thus, it was the purpose of this investigation to identify compounds that would selectively modify the pressor effects of angiotensin, either singly or in combination, in order to elucidate more clearly the mode of action of angiotensin in this system.

MATERIALS AND METHODS

Full-term human placentas, obtained from the hospital 15–20 min. after normal delivery, were used throughout this investigation. Each was transported to the laboratory in a light-resistant glass container filled with 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 (3, 6). In 32 successful placental preparations, each lasting 1–4 hr., a total of 116 studies was performed. The results from three 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 test compounds for the purpose of establishing a standard response to its vasoconstrictor effect. 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 (1). This dose of angiotensin produced a mean increase in perfusion pressure of 20.3 mm. Hg (range 10.6–34.0 and $SD \pm 6.6$). The test compounds were administered 2–3 min. after the pressor response to angiotensin subsided. Subsequent responses to angiotensin, at 4–6 min. intervals after the various pressor or depressor effects of the test compounds waned, were then compared to the initial standard. The mean pressure change, measured at maximal effectiveness of the test agents, as well as the mean onset of action in minutes and the percent change at maximal efficacy, were used as the bases for comparing the relative potencies of the compounds tested.

The doses of the test agents were determined as in a previous study (7) and were kept as low as possible yet sufficient to affect the pressor response to angiotensin. However, in the case of the α-adrenergic blocking agents, their doses were based on those used by Mancini and Gautieri (3) to antagonize the constrictor effect of a 40.0-mcg. dose of norepinephrine.

The following drugs were injected into the rubber tubing, prior to entering the pump (Sigmamotor), in a volume of distilled water not exceeding 5.0 ml. except where noted: angiotensin-II,[1] 0.01%; nitroglycerine USP, 0.1%; sodium nitrite, 0.4%; dipyridamole,[2] 0.5% in an aqueous solution containing polyethylene glycol 600, 50 mg., and tartaric acid, 2.0 mg./ml.; phenoxybenzamine hydrochloride,[3] 0.2% in USP alcohol; phentolamine methanesulfonate,[4] 0.6%; tolazoline hydrochloride,[5] 0.2%; hydralazine hydrochloride,[6] 0.2%; isoproterenol hydrochloride,[7] 0.1%; pronethalol hydrochloride,[8] 0.1 and 0.2%; and dichloroisoproterenol hydrochloride (DCI), 0.1 and 0.2%.

Statistical data presented in the tables, including the standard error of the mean (*SEM*) and the *p* value, were calculated from the paired *t* test (8).

RESULTS

The following results, which are summarized in Tables I and II, were obtained on the vasculature of full-term human placentas perfused at pressures between 70 and 100 mm. Hg. This range of perfusion pressures corresponds to inflow rates of 41–62 ml. of perfusion fluid (Tyrode's solution modified by the addition of 0.525% polyvinylpyrrolidone[9]) per minute.

Nitroglycerine—The 4.0-mg. dose of nitroglycerine was one of the more potent angiotensin antagonists employed, as evidenced by a mean decrease in perfusion pressure of 55.8% at maximal antagonism. The onset of maximal antagonism was 4.4 min. on the average; and in each of the five experiments performed, nitroglycerine itself produced a decrease in perfusion pressure ranging from 4.2 to 12.0 mm. Hg (mean of 7.1 mm. Hg).

Sodium Nitrite—In a total of six experiments, an 8.0-mg. dose of sodium nitrite produced a mean decrease in the pressor response to angiotensin of 38.5% at maximal efficacy. The mean onset of antagonism was 11.3 min. and in each study, sodium nitrite itself produced a mean drop in perfusion pressure of 6.1 mm. Hg (range 3.6–11.0). On the kymograph record, this drop was always seen before angiotensin was administered.

Dipyridamole—From Table I it can be seen that 2.5 mg. of dipyridamole produced a mean decrease in the pressor response to

[1] Hypertensin, valyl-5-angiotensin-II amide, lot No. B-5578, supplied through the courtesy of Ciba Pharmaceutical Co., Summit, N. J.

[2] Persantin, supplied through the courtesy of Geigy Chemical Corp., Ardsley, N. Y.

[3] Dibenzyline, supplied through the courtesy of Smith, Kline and French Labs., Philadelphia, Pa.

[4] Regitine, supplied through the courtesy of Ciba Pharmaceutical Co., Summit, N. J.

[5] Priscoline, supplied through the courtesy of Ciba Pharmaceutical Co., Summit, N. J.

[6] Apresoline, supplied through the courtesy of Ciba Pharmaceutical Co , Summit, N. J.

[7] Isuprel, supplied through the courtesy of Sterling-Winthrop Research Institute, Rensselaer, N. Y.

[8] Alderlin, supplied through the courtesy of Imperial Chemical Industries, Ltd., Wilmslow Cheshire, Great Britain.

[9] Plasdone C; povidone.

Table I—Comparison of Angiotensin Antagonists in Perfused Human Placental Blood Vessels

Drug	Dose, mg.	No. of Expt.	Mean Angiotensin Pressure Increase before Antagonist, mm. Hg	Av. Max. Antagonism, min.	Mean Pressure Change, mm. Hg	*SEM*	*p* Value[a]	Mean % Pressure Change
Nitroglycerine	4.0	5	10.3	4.4	−5.84	±1.04	0.005	−55.8
Sodium nitrite	8.0	6	20.3	11.3	−7.53	±1.27	0.005	−38.5
Dipyridamole	2.5	5	18.1	7.4	−7.84	±1.78	0.010	−42.4
Phenoxybenzamine	2.0	5	16.8	17.2	−4.92	±1.25	0.010	−30.2
Phenoxybenzamine	4.0	5	17.3	14.2	−5.64	±1.36	0.010	−35.8
Phentolamine	6.0	5	12.0	9.0	−6.44	±1.63	0.010	−51.1
Phentolamine	12.0	5	16.1	3.2	−6.20	±1.01	0.005	−38.2
Tolazoline	10.0	5	18.6	5.0	−10.40	±2.67	0.010	−55.7
Hydralazine	8.0	5	19.4	5.8	−11.44	±2.87	0.010	−57.7
Isoproterenol	0.25	5	18.7	7.2	−6.40	±1.68	0.010	−35.7
Isoproterenol	0.5	5	22.7	6.8	−8.92	±1.96	0.010	−39.6
Isoproterenol	1.0	5	20.4	4.2	−6.64	±0.54	0.0005	−35.2
Isoproterenol	2.0	5	17.1	5.2	−5.76	±0.83	0.0005	−34.7
Isoproterenol Phentolamine	0.25 6.0	5	14.7	6.4	−10.44	±1.46	0.005	−71.6
Isoproterenol Tolazoline	0.25 10.0	5	17.2	7.8	−11.60	±1.57	0.005	−68.3
Isoproterenol Hydralazine	0.25 8.0	5	13.2	5.9	−9.48	±1.60	0.005	−70.6
Isoproterenol Nitroglycerine	0.25 4.0	5	11.0	4.4	−8.24	±1.65	0.005	−77.7
Nitroglycerine Tolazoline	4.0 10.0	5	10.6	4.2	−7.36	±1.02	0.005	−69.1

[a] *p* Value > 0.050 not significant.

angiotensin of 42.4% at maximal antagonism which occurred at an average of 7.4 min. after this antagonist had been administered. Administration of dipyridamole consistently was followed by a mean drop in perfusion pressure of 4.9 mm. Hg (range 1.4–9.4).

Phenoxybenzamine—The larger (4.0 mg.) dose of phenoxybenzamine used was the more potent angiotensin antagonist, producing a mean decrease in perfusion pressure of 35.8% at maximal antagonism. The onset of maximal antagonism was shorter with the larger dose (14.2 *versus* 17.2). Each administration of phenoxybenzamine itself produced a mean rise in the perfusion pressure which was related to the dose administered (+6.6 mm. Hg for the 2.0-mg. dose and +20.5 mm. Hg for the 4.0-mg. dose.)

Phentolamine—In a total of 10 experiments it can be seen that the smaller (6.0 mg.) dose of phentolamine produced a greater decrease in the pressor response to angiotensin than did the larger (12.0 mg.) dose, *i.e.*, a mean decrease in the pressor response to angiotensin of 51.1 *versus* 38.2%, respectively. The larger dose, however, had a more rapid onset of action, 3.2 *versus* 9.0 min. at maximal efficacy. Phentolamine had no consistent effect on perfusion pressure itself, regardless of the dose; its administration was followed by changes in perfusion pressure that ranged from −2.0 to no effect to +1.8 mm. Hg.

Tolazoline—From Table I it can be seen that the 10.0-mg. dose of tolazoline produced a mean decrease in perfusion pressure of 55.7% at maximal antagonism. It was one of the more potent angiotensin antagonists tested and had a mean onset of action of 5.0 min. The administration of tolazoline was usually followed by a mean drop in perfusion pressure of 2.0 mm. Hg although in one experiment an increase of 1.8 mm. Hg was noted.

Hydralazine—The 8.0-mg. dose of hydralazine was the most potent of the α-adrenergic blocking agents tested, reducing the pressor response to angiotensin an average of 57.7% at maximal effectiveness. Its onset of action was 3.2 min. on the average and each dose of hydralazine was immediately followed by a mean drop in the perfusion pressure of 6.2 mm. Hg (range 2.6–12.8.)

Isoproterenol—In a total of 20 experiments, the 0.5-mcg. dose of isoproterenol was found to be most effective, reducing the pressor response to angiotensin an average of 39.6%. The onset of maximal antagonism decreased as the dose increased and ranged from 7.2 min. for the 0.25-mg. dose to 5.2 min. for the 2.0-mg. dose. No such relationship was noted when the mean percent pressure change was used as the basis for comparion; in fact, all doses were approximately equipotent. Administration of the antagonist was always followed immediately by a reduction in perfusion pressure that ranged from 2.4 to 12.4 mm. Hg.

Isoproterenol and Phentolamine—A combination of 0.25 mg. of isoproterenol and 6.0 mg. of phentolamine antagonized the pressor effect of angiotensin an average of 71.6%. This percent decrease was greater than that seen with either antagonist alone, but was less than the sum of the individual actions. The onset of maximal antagonism (6.4 min.) was somewhat less than that of either of the drugs when used separately.

Isoproterenol and Tolazoline—From Table I it can be seen that a combination of 0.25 mg. of isoproterenol and 10.0 mg. of tolazoline

Table II—Augmentation of the Pressor Effect of Angiotensin in Perfused Human Placental Blood Vessels

Drug	Dose, mg.	No. of Expt.	Mean Angiotensin Pressure Increase before Antagonist, mm. Hg	Av. Max. Augmentation, min.	Mean Pressure Change, mm. Hg	*SEM*	*p* value[a]	Mean Percent Pressure Change
Pronethalol	2.5	5	14.8	6.0	+0.88	±0.78	0.200[a]	+9.2
Pronethalol	5.0	5	16.0	7.2	+3.96	±0.63	0.005	+29.5
Pronethalol	10.0	5	11.6	10.0	+4.20	±0.74	0.005	+35.6
DCI	5.0	5	18.7	4.9	+1.60	±1.32	0.150[a]	+6.1
DCI	10.0	5	18.1	7.6	+5.24	±3.31	0.100[a]	+24.3

[a] *p* value > 0.050 not significant.

reduced the pressor response to 50.0 mcg. of angiotensin an average of 68.3%. This reduction in the response to angiotensin was greater than that seen with either drug alone, but was less than the sum of the drugs when tested individually. The onset of maximal antagonism (7.8 min.) was similar to that seen with the 0.25-mg. dose of isoproterenol alone (7.2 min.) and somewhat greater than that seen with tolazoline alone (5.0 min.)

Isoproterenol and Hydralazine—In a total of five experiments, a combination of 0.25 mg. of isoproterenol and 8.0 mg. of hydralazine reduced the pressor response to 50.0 mcg. of angiotensin an average of 70.6%. This degree of antagonism was greater than seen with either of the antagonists used separately but somewhat less than the sum of their individual results. The onset of maximal antagonism with the combination (5.9 min.) was similar to that seen with hydralazine alone but less than that of isoproterenol.

Isoproterenol and Nitroglycerine—A combination of 0.25 mg. of isoproterenol and 4.0 mg. of nitroglycerine produced an average decrease in the pressor response to subsequent doses of angiotensin of 77.7%. The response to the drug combination was greater than the action of either drug alone but was less than the sum of their individual actions. The onset of maximal antagonism (4.4 min.) was similar to that recorded for 4.0 mg. of nitroglycerine but was smaller than that seen with isoproterenol.

Nitroglycerine and Tolazoline—In a total of five experiments, a combination of 4.0 mg. of nitroglycerine and 10.0 mg. of tolazoline antagonized the pressor action of 50.0 mcg. of angiotensin an average of 69.1% (Table I). This response to the combination of antagonists was greater than the action of the two antagonists tested separately, but much less than the sum of their individual actions. The onset of maximal antagonism (4.2 min.) was similar to that seen when the two drugs were tested separately.

Pronethalol—From Table II it can be seen that the 10.0-mg. dose of pronethalol was the most potent angiotensin potentiator tested, increasing the pressor response to 50.0 mcg. of angiotensin an average of 35.6%; this dose of pronethalol was, however, only slightly more effective than the 5.0-mg. dose, which produced a pressure increase after angiotensin of 29.5%. The onset of maximal efficacy occurred an average of 10.0 min. after the 10.0-mg. dose and did not seem to be dose-dependent. Administration of the higher doses of this agent was usually followed by a decrease in perfusion pressure that ranged from 1.2 to 12.8 mm. Hg; the lowest dose of pronethalol, 2.5 mg., had little or no effect on perfusion pressure. There was a correlation between the dose of pronethalol and the degree of angiotensin potentiation, with the lowest dose being least effective (Table II).

Dichloroisoproterenol—In a total of 10 experiments, the 10.0-mg. dose of DCI was found to be the more effective angiotensin potentiator, increasing the pressor response to 50.0 mcg. of the agonist an average of 24.3%, or about four times as much as the lower dose. The onset of maximal potentiation (7.6 min.) was somewhat greater with the larger dose, and in most cases, administration of DCI itself produced a decrease in perfusion pressure that ranged from 1.2 to 5.6 mm. Hg (Table II).

DISCUSSION

The majority of drugs tested as angiotensin antagonists in this investigation were chosen from two pharmacologic classes: negative musculotropic agents (nitroglycerine, sodium nitrite, and dipyridamole) and compounds that selectively block α-adrenergic receptors (phenoxybenzamine, phentolamine, tolazoline, and hydralazine.)

Among the direct-acting smooth muscle depressants, nitroglycerine (4.0 mg.), was the most potent compound tested, producing a mean decrease in the pressor response to angiotensin of 55.8%. Each of the drugs in this category also produced a mild and transient decrease in perfusion pressure which was allowed to subside before its ability to antagonize the pressor response to angiotensin was tested. Tolazoline (10.0 mg.) and hydralazine (8.0 mg.) were the most potent α-adrenergic blocking agents used in this study, antagonizing the pressor response to angiotensin an average of 55.7 and 57.7%, respectively. Little difference in potency could be noted between the two groups of antagonists, with the α-adrenergic blocking agents being slightly more effective. The reported dual mechanism of action for phentolamine, tolazoline, and hydralazine (9), which involves blockade of α-adrenergic receptors as well as direct depression of vascular smooth muscle, may offer an explanation for the increased potency of this group of antagonists. No explanation is yet forthcoming for the observation that doubling the dose of phentolamine decreased its antagonistic ability. With phenoxybenzamine, doubling the dose increased the degree of angiotensin antagonism only slightly. Perhaps autoinhibition or poisoning of the vascular receptors offers a plausible explanation. In the case of phenoxybenzamine, the pressure increase that immediately followed its administration has been attributed to the small volumes of 95% ethyl alcohol that were used as the vehicle (1). These studies confirm other reports (1, 2) which have suggested that the pressor effect of angiotensin in these vessels is the result of both stimulation of α-adrenergic receptors as well as direct stimulation of vascular smooth muscle.

In addition to the above drugs, isoproterenol (a potent stimulant of β-adrenergic receptors) was also tested as an angiotensin antagonist because previous studies with this preparation (3, 10) have shown that β-adrenergic receptors are present in the placental blood vessels. Although the 0.5-mg. dose of this antagonist was most effective, as with phenoxybenzamine and phentolamine, the differences in potency between the smallest and largest dose was not significant.

Combinations of certain drugs were also tested as angiotensin antagonists (Table I). The degree of angiotension antagonism demonstrated with the combination of antagonists, although less than the sum of the individual agents, did approach this value. No potentiation or synergism was noted while testing these combinations of antagonists. These results again demonstrated the duplicity of mechanisms involved in the pressor effect of angiotensin in the placental vessels.

The β-adrenergic blocking agents, pronethalol and DCI, were also tested for their effect on the pressor response to angiotensin in this preparation (Table II). Both agents, in a dose of 10.0 mg., augmented the pressor response to angiotensin an average of 35.6 and 24.3%, respectively. This was to be expected in view of the work of Vogin and Buckley (5) who augmented the pressor effect of angiotensin with similar compounds in the anesthetized dog. The greater potency of pronethalol over DCI has been attributed to a greater inherent stimulation of α-receptors by DCI, offsetting the β-blockade (11). Therefore, the results with pronethalol and DCI suggest a third mode of action for angiotensin. In addition to stimulating α-receptors and vascular smooth muscle, angiotensin may also stimulate β-receptors, offsetting its other two actions. Thus, when the β-adrenergic receptors are blocked, the pressor response to angiotensin is augmented.

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