Effect of Certain Drugs on Perfused Human Placenta III

Sympathomimetics, Acetylcholine, and Histamine

By HENRY P. CIUCHTA and RONALD F. GAUTIERI

Perfusion studies conducted on full term human placentas indicated that histamine and serotonin consistently cause marked increases in perfusion pressure. A moderate increase in pressure was observed in response to epinephrine and *l*-epinephrine. In addition, norepinephrine and acetylcholine produced only slight increases in the perfusion pressure. Maximum effects were recorded with the above agents when the preparation was composed of a long cord placenta. Iproniazid was incapable of enhancing the action of the sympathomimetic amines tested significantly.

IT IS WELL KNOWN that the neurohormones, either in normal homeostatic concentrations or in response to drug therapy, are the controlling factors in bringing about the overall integrity of many body functions. However, an upset in the balance of any of the naturally occurring mediators—epinephrine, norepinephrine, acetylcholine, and serotonin—as well as histamine production, may result in the occurrence of cardiovascular disease, metabolic disturbances, and even mental aberrations. When an imbalance of any of these neurohormones is incurred during pregnancy, even greater complications arise which endanger maternal and fetal life.

It has been postulated by Luschinsky and Singher (1) that increased levels of pressor amines, resulting from changes in monoamine oxidase activity in the placenta, may be a causative factor in the development of toxemia of late pregnancy. In a subsequent investigation (2) Luschinsky rejected this theory. Eliasson and Astrom (3) also proved this concept inadequate by *in vitro* placental studies that indicated epinephrine and norepinephrine concentrations were not enhanced to an appreciable degree by the monoamine oxidase inhibitors, propamidine and *l*-isonicotinyl-2-isopropyl hydrazine (IIH).

The effects of the sympathomimetic amines, acetylcholine, serotonin, and histamine, on vessels of the perfused human placenta have been investigated quite extensively. There is agreement regarding the vasoconstrictor activities of serotonin (3–5, 7–9) and histamine (3, 4, 6, 10, 11), but the same cannot be said for the effects of epinehrine, *l*-epinephrine, norepinephrine, and acetylcholine.

Early perfusion experiments conducted by Schmitt (10, 12) established that adrenaline lacked vasoconstrictor activity on isolated placental vessels because of the absence of sympathetic fibers in the vasculature. Subsequent placental perfusion experiments conducted by Kosakae (13), Budelmann (14), Ueda (15), Ordynsky (16), Eliasson and Astrom (3), Astrom and Samelius (9), and Panigel (6) demonstrated that adrenaline possessed vasoconstrictor activity. However, a great degree of variance existed quantitatively. von Euler (11) confirmed Schmitt's original finding that adrenaline did not act on individually isolated placental vessels, but also reported that perfusion of the whole placental preparation elicited vasoconstriction after adrenaline administration. These studies also demonstrated that acetylcholine had weak vasoconstrictor activity. However, previously it had been observed by Ueda (15) that low concentrations of acetylcholine caused dilatation, while higher concentrations produced vasoconstriction of placental vasculature. The inconsistency of acetylcholine's action has been confirmed by Eliasson and Astrom (3), Panigel (5, 6), and others.

Nyberg and Westin's experiments (17) with placental perfusions indicated that noradrenaline produced an increase in perfusion pressure. Eliasson and Astrom (3) observed similar activity with noradrenaline but noted, as did Panigel (5, 6), that a greater degree of constriction occurred in placental preparations with long umbilical cords (8–20 cm.) than in those with relatively short cords (0–3 cm.).

Although the actions of some neurohormones

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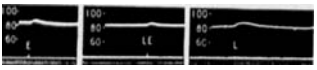

Fig. 1.—Typical pressure responses in short cord placental preparations. Key: E = epinephrine HCl (10–20 mcg.); LE = Levophed bitartrate (10–20 mcg.); L = *l*-epinephrine bitartrate (10–20 mcg.).

on placental vessels appear relatively weak, Dornhorst and Young reported (18), with *in situ* experiments on rabbit and guinea pig placentas, that epinephrine and norepinephrine may act upon maternal-placental circulation producing constriction and thus impairing placental blood flow with a subsequent production of fetal asphyxia. Although a correlation of these observations cannot be made with human placentas at this time, subsequent investigations may amplify this problem.

Since the placenta is devoid of innervation (10, 12, 15, 19), it serves as a useful preparation in ascertaining drug activity without the influence of neurotropic activity. Its application in determining the musculotropic action of drugs on vasculature has been indicated in a previous paper (8). Consequently, in view of the irregular responses obtained with epinephrine, *l*-epinephrine, levarterenol, and acetylcholine, an investigation was conducted to determine the effects of these agents and to confirm serotonin and histamine activity on isolated vessels of human placentas perfused at various pressures and having either long or short umbilical cords.

MATERIALS AND METHODS

Full term human placentas were obtained from the hospital 5–15 minutes after normal delivery. Each was transported to the laboratory in a container of preheated (38°) Tyrode's solution.

The preparation was flushed free of blood by injecting 2.3% sodium citrate solution into one of the umbilical arteries. Cannulae were inserted into the vein and one artery of the umbilical cord. The umbilical cord was either 2–3 cm. (short cord) or

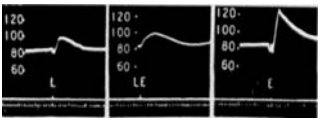

Fig. 2.—Typical pressure responses in long cord placental preparations. Key: L = *l*-epinephrine bitartrate (10–20 mcg.); LE = Levophed bitartrate (10–20 mcg.); E = epinephrine HCl (10–20 mcg.).

8–10 cm. (long cord) from the stalk of the placenta, depending on how much of the cord was free of clots and constrictions. The remaining umbilical artery was ligated. The whole preparation was then placed into a perfusion chamber containing aerated Tyrode's solution which had a pH range of 7.2 to 7.4 and was maintained at a constant 37° with a Bronwill thermoregulator. The arterial cannula was connected with a short piece of rubber tubing to polyethylene tubing which passed through a model TS-8 Sigmamotor pump attached to a 5-gal. polyethylene reservoir. The reservoir contained aerated Tyrode's solution which was modified by the addition of 0.525% polyvinylpyrrolidone (Plasdone C) and kept at a constant 40° by another Bronwill thermoregulator.

Adjustment of the Sigmamotor pump produced constant perfusion pressures which were set between 50–90 mm. Hg and were accompanied by volume inflow rates of 35–75 ml. per minute. Volume inflow was measured by a glass RGI flowmeter placed between the pump and arterial cannula; perfusion pressure was recorded by a mercury manometer located between the pump and flowmeter. Recordings of pressure fluctuations were

TABLE I.—RESPONSES OBTAINED WITH ISOLATED HUMAN PLACENTAS PERFUSED AT 50–90 mm. Hg PRESSURE

Drug Dose, mcg.	Cord Length[a]	Range of Pressure Changes, mm. Increase	(No. expt.)	Decrease	(No.) expt.	Av. Increase, mm.	Unresponsive Experiments, No.
Epinephrine, 10–20	S	2–5	3	2	1	3.3	...
	L	4–39	5	...	...	12.0	1
Epinephrine, 200–500	S	3	1	...	...	...	1
	L	4–6	2	1–2	2	...	...
Levarterenol, 10–20	S	1–5	5	...	...	2.8	...
	L	1–14	5	...	...	4.6	2
Levarterenol, 200–500	S	1–7	2	...	...	...	...
	L	1–4	3	...	...	2.3	...
l-Epinephrine, 2	S	4–12	2	...	...	...	...
	L	1–10	5	...	...	5.8	1
l-Epinephrine, 10–20	S	3–10	5	11	1	5.8	...
	L	3–16	4	...	...	10.8	...
Acetylcholine, 100	S	1–5	8	...	...	2.6	2
Histamine, 10–20	S	5–50	8	...	...	14.1	1
	L	5–192	9	5–8	2	46.1	...
Histamine, 100	L	28–100	3	...	...	65.3	...
Histamine, 1000	S	...	...	11	1	...	...
	L	52–136	4	...	...	92.5	...
Serotonin, 2–4	S	3–54	4	...	...	21.3	1
	L	6–168	6	...	...	90.0	...
Serotonin, 50	S	8–76	15	...	...	30.2	...
	L	17–180	4	...	...	76.5	...

[a] S = small cord; L = long cord.

TABLE II.—COMPARATIVE RESPONSES BETWEEN NORMAL AND IPRONIAZID TREATED VESSELS OF ISOLATED HUMAN PLACENTAS WITH SHORT UMBILICAL CORDS AND PERFUSED AT 50–90 mm. Hg PRESSURE

Drug Dose, mcg.	Range of Pressure Changes, mm. Increase	(No. expt.)	Decrease	(No. expt.)	Av. Increase, mm.	Unresponsive Experiments, No.
Epinephrine, 20						
before iproniazid	2–5	3	2	1	3.3	...
after iproniazid	2–6	5	...	...	3.4	...
Levarterenol, 20						
before iproniazid	1–5	5	...	...	2.8	...
after iproniazid	2–4	3	...	...	3.0	2
l-Epinephrine, 20						
before iproniazid	3–10	4	11	1	5.8	...
after iproniazid	3–14	6	...	...	6.1	2
Acetylcholine, 100						
before iproniazid	1–5	8	...	...	2.6	2
after iproniazid	2–5	3	...	...	3.3	3
Histamine, 20						
before iproniazid	5–50	8	...	...	14.1	1
after iproniazid	15–60	6	2–5	2	25.8	1
Serotonin, 50						
before iproniazid	8–76	15	...	...	30.2	...
after iproniazid	12–66	10	...	...	32.6	...

made on the smoked drum of a Livingston long paper kymograph. The venous volume outflow, which was measured directly by a graduated glass cylinder and never reperfused, was determined at the onset of each experiment and at various intervals in order to compare it with the volume inflow. In 41 successful placental preparations, each lasting 1–4 hours, a total of 211 different experiments were performed. The results obtained from nine placentas were discarded because the volume inflow at the onset of the experiments was below average, or placental perfusion pressure could not be maintained at a constant level.

The following agents were injected into the polyethylene tubing prior to entering the pump in a volume of distilled water not exceeding 1 ml.: acetylcholine hydrobromide, 1.0%; histamine dihydrochloride, 0.1%; 5-hydroxytryptamine creatinine sulfate (serotonin), 0.01%; epinephrine hydrochloride, 0.1%; *l*-epinephrine bitartrate, 0.1%; levarterenol bitartrate, 0.2% (0.1% as base); and iproniazid, 1.0%.

RESULTS

The following results were obtained from placental preparations having umbilical cords 2–3 cm. long and perfused at a pressure of 50–90 mm. Hg and a volume inflow of 35–75 ml. per minute, unless stated otherwise.

Epinephrine.—Doses of 10–20 mcg. of epinephrine hydrochloride brought about an increase in perfusion pressure (Fig. 1*a*) in three cases, having a range of 2–5 mm. (av. 3.3 mm.). One experiment demonstrated a 2-mm. decrease in pressure. A dose range of 200–500 mcg. produced a 3-mm. increase in one experiment and no response in another (Table I).

Epinephrine, in doses of 10–20 mcg., produced a 4–39-mm. (av. 12.0 mm.) increase in pressure (Fig. 2*c*) in five preparations where the umbilical cord was 8–10 cm. long. No response occurred in one experiment (Table I); an increase in pressure followed by a decrease was observed in two cases. The administration of 200–500 mcg. of epinephrine caused an increase in pressure in two experiments and a decrease in pressure in two others (Table I).

The injection of 5 mg. iproniazid[1] prior to the administration of 20 mcg. epinephrine elicited an increase in perfusion pressure of 2–6 mm. (av. 3.4 mm.) in five experiments (Table II).

Levarterenol.—Levarterenol bitartrate, administered in doses of 10–20 mcg. in five placentas produced an increase in pressure (Fig. 1*b*), having a range of 1–5 mm. (av. 2.8 mm.). A dose range of 200–500 mcg. caused an increase of 1 and 7 mm. in two cases (Table I), while two experiments exhibited an increase in pressure followed by a decrease.

Employing placentas with cords 8–10 cm. long, the administration of 10–20 mcg. levarterenol caused a 1–14-mm. (av. 4.6 mm.) increase in pressure (Fig. 2*b*) in five preparations. No response was observed in two cases. Doses of 200–500 mcg. produced a 1–4-mm. (av. 2.3 mm.) increase in perfusion pressure in three cases (Table I).

In five experiments, pretreatment with 5 mg. of iproniazid, followed by 20 mcg. of levarterenol elicited an increase of 2–4 mm. (av. 3.0 mm.) in three cases. No response was obtained in the other two (Table II).

l-Epinephrine[2] Bitartrate.—A dose of 2 mcg. of *l*-epinephrine bitartrate caused an increase in perfusion pressure in two experiments. A dose range of 10–20 mcg. in five cases produced a 3–10-mm. (av. 5.8 mm.) increase (Fig. 1*c*), while a decrease of 11 mm. was recorded in another experiment (Table I).

In placental preparations with cords 8–10 cm. long, a dose of 2 mcg. of *l*-epinephrine caused a 1–10-mm. (av. 5.8 mm.) increase in perfusion pressure in five cases, while no response was observed in one experiment. Doses of 10–20 mcg. of *l*-epinephrine in four preparations produced an increase in pressure (Fig. 2*a*), having a range of 3–16 mm. (av. 10.8, Table I).

The prior administration of 5 mg. of iproniazid in six placentas receiving 10–20 mcg. *l*-epinephrine elicited a 3–14-mm. (av. 6.1 mm.) increase in per-

[1] Supplied through the courtesy of Hoffmann-LaRoche, Inc., Nutley, N. J.

[2] Supplied through the courtesy of Sterling-Winthrop Research Institute.

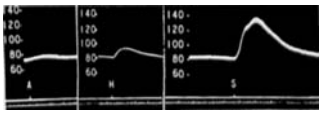

Fig. 3.—Typical pressure responses in short cord placental preparations. Key: A = acetylcholine HBr (100 mcg.); H = histamine dihydrochloride (10–20 mcg.); S = serotonin (50 mcg.).

fusion pressure. Two other placental preparations were unresponsive (Table II).

Acetylcholine.—In eight experiments the administration of 100 mcg. of acetylcholine hydrobromide evoked a 1–5-mm. (av. 2.6 mm.) increase in perfusion pressure (Fig. 3*a*), while two cases were unresponsive (Table I).

The injection of 5 mg. of iproniazid, followed by 100 mcg. of acetylcholine caused an increase in pressure in three cases, having a range of 2–5 mm. (av. 3.3 mm.). Three other preparations did not elicit a response (Table II).

Histamine.—Doses of 10–20 mcg. of histamine dihydrochloride in eight experiments produced an increase in pressure (Fig. 3*b*) having a range of 5–50 mm. (av. 14.1 mm.). One placenta did not respond. A 1000-mcg. dose of histamine produced a decrease in the only case tested (Table I).

The administration of 10–20 mcg. of histamine in nine experiments with long cords resulted in a 5–192-mm. (av. 46.1 mm.) increase in perfusion pressure (Fig. 4*b*). Pressure decreases of 5 and 8 mm. were observed in two cases. An increase in pressure followed by a decrease was noted in two experiments. A dose of 100 mcg. of histamine produced a 28–100-mm. (av. 65.3 mm.) increase in perfusion pressure in three experiments. Histamine, in a dose of 1000 mcg., caused an increase (Fig. 4*c*) in pressure in four cases, having a range of 52–136 mm. (av. 92.5 mm.) (Table I).

The administration of 5 mg. of iproniazid prior to histamine resulted in an increase in perfusion pressure in six cases. The range of the increase was 15–60 mm. (av. 25.8 mm.). One preparation did not respond, and two others demonstrated decreases in pressure (Table II).

Serotonin.[a]—The administration of 2–4 mcg. of serotonin in four experiments brought about a 3–54-mm. (av. 21.3 mm.) increase in pressure, while one other placenta was unresponsive. A dose of 50 mcg. of serotonin (15 cases) caused an increase in pressure (Fig. 3*c*), having a range of 8–76 mm. (av. 30.2 mm.) (Table I).

Serotonin, in doses of 2–4 mcg., caused a 6–168-mm. (av. 90 mm.) increase (Fig. 4*a*) in perfusion pressure in six experiments where the umbilical cord was 8–10 cm. long. A dose of 50 mcg. of serotonin in four experiments produced a 17–180-mm. (av. 76.5 mm.) increase in pressure (Table I).

The injection of 5 mg. of iproniazid preceding 50 mcg. of serotonin evoked a 12–66-mm. (av. 32.6 mm.) increase in pressure in ten preparations (Table II).

Perfusion experiments attempted with pressures less than 50 mm. Hg—hence with lower volume inflow rates—resulted in irregular and inconsistent responses with the above drugs in the same dose ranges.

[a] Supplied through the courtesy of Abbott Laboratories, North Chicago, Ill.

DISCUSSION

The results of this investigation have indicated that the administration of the sympathomimetic amines—epinephrine, *l*-epinephrine, and levarterenol—and acetylcholine, histamine, and serotonin, to vessels of perfused human placentas caused vasoconstriction in practically all preparations with short (2–3 cm.) or long (8–10 cm.) umbilical cords.

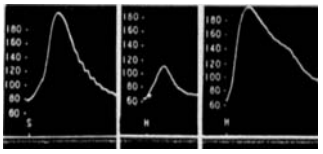

Fig. 4.—Typical pressure responses in long cord placental preparations. Key: S = serotonin (2–4 mcg.); H = histamine dihydrochloride (10–20 mcg.); H = histamine dihydrochloride (1000 mcg.).

In most cases the degree of vasoconstriction produced was greater in the preparations with long cords. This increased responsiveness of long cord compared to short cord preparations also has been reported by Eliasson and Astrom (3). Though direct evidence is lacking at this time, it appears likely that the vascular musculature present in the umbilical cord may have greater amounts of responsive effector cells than other placental vessels. Hence, when a relatively long cord is present in a placental perfusion, it contributes more to the overall vasoconstriction than the other placental vessels.

The responses obtained with the amines and acetylcholine in long and short cord preparations were not in direct proportion to the doses administered, since low (10–20 mcg.) or high (200–500 mcg.) doses usually elicited responses that did not vary quantitatively in the same perfusion. von Euler (11) has also pointed out that a dose-response relationship is seemingly nonexistent in placental perfusions. This fact was confirmed in these studies, for vascular responsiveness depended upon the presence of the agent and not its quantity. However, histamine was the exception in this respect, as larger doses based on average response produced greater increases in perfusion pressure.

Other investigations (3) have demonstrated that *l*-epinephrine and levarterenol produce consistently marked increases in perfusion pressure, although levarterenol is the least potent. This investigation did not corroborate the marked constrictor action of these amines, but did agree in finding levarterenol less effective than epinephrine or *l*-epinephrine in causing vasoconstriction of placental vessels.

The relative lack of activity of the sympathomimetic amines and acetylcholine on vessels of human placentas was expected, since the placenta is devoid of innervation, and its vessels probably have a correspondingly fewer effector cells than other vasculature.

Eliasson and Astrom (3) and von Euler (11) obtained inconsistent results with acetylcholine on placental vasculature, although slight vasoconstrictor activity usually was predominant. It was proposed, from von Euler's investigation (20) on pulmonary vessels of the rabbit, that a correlation may exist between the response of placental and pulmonary vessels because both constrict in response to acetyl-

choline, whereas other vascular beds are dilated. Results in this present investigation confirm that acetylcholine produces weak vasoconstriction in placental vessels—and sometimes even lacks this action.

Pretreatment of placental preparations with 5 mg. of iproniazid (only short cord placentas were tested) before the administration of the sympathomimetic amines, acetylcholine, or serotonin, did not produce an appreciable augmentation of perfusion pressure—except in the case of histamine—which at this time is inexplicable. However, increases in sympathomimetic amine activity would be expected since monoamine oxidase is one of the enzymes involved in the breakdown of the amines, and blockage of this placental enzyme sometimes can produce slight increased amine activity. In view of the results, it is possible that iproniazid is washed out too rapidly to tie up effectively the large quantity of monoamine oxidase present in the placenta. Also, it is possible that monoamine oxidase is so abundant that whatever quantity is blocked is still not sufficient to allow augmented responses to occur with these amines. These results are similar to studies conducted by Eliasson and Astrom (3), who showed that increased responses do not occur after administration of *l*-epinephrine or levarterenol to propamidine and *l*-isonicotinyl-2-isopropyl hydrazine (IIH) treated placental preparations.

Perfusion experiments attempted at lower pressures (under 50 mm. Hg), and consequently lower volume inflow but the same dose ranges, produced only slight changes in perfusion pressure with all the previously mentioned agents. Therefore, there appears to be a dependence upon volume inflow and perfusion pressure for the attainment of significant responses. Optimum results were recorded when the volume inflow rate was at least 70 ml. per minute, and the perfusion pressure was at least 60 mm. Hg, but preferably around 80 mm. Hg.

Although the results reported here are those occurring *in vitro*, the possibility of similar qualitative effects being manifested *in vivo* with the above drugs cannot be overlooked. With this in mind, it appears likely that the systemic release of excess quantities of sympathomimetic substances, serotonin, or even histamine in the pregnant state could, by directly constricting placental vessels, cause a decrease in the oxygenation of fetal blood flowing through such vessels—and hence contribute to fetal asphyxia.

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Properties of Fused Mannitol in Compressed Tablets

By JOSEPH L. KANIG

Among the carbohydrates used in compressed tablets, mannitol is the only one which possesses high heat stability. This substance melts at 167°, but does not decompose at temperatures up to 250°. Mannitol, alone and in combination with other carbohydrates, was fused and recrystallized or spray-congealed. Phase diagrams determined for these mixtures indicated that mannitol is eutectic with other carbohydrates. Selected drugs were soluble in the fused mixtures, and the crystallized or spray-congealed material obtained from these solutions possessed excellent flow and compression characteristics.

MANNITOL HAS BEEN used as a tablet diluent for more than a decade. This hexahydric alcohol, an isomer of sorbitol, is a white, odorless, crystalline powder and is the least hygroscopic of all known carbohydrate tablet diluents (1). It has a sweetness threshold of approximately the same value as glucose and a nutritive value of 2 cal. per gram (2).

In recent years, mannitol has been shown to exhibit a uniquely cooling and pleasant taste effect when used in formulations for tablets intended to be chewed or dissolved in the mouth. Several types of medicaments, including antacids, analgesics, multivitamins, and antihistamines are being marketed in the form of chewable tablets prepared in a mannitol base (3, 4). This diluent

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