Effect of Certain Drugs on Perfused Human Placenta X: Norepinephrine Release by Bradykinin

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Abstract □ Norepinephrine was detectable chemically in the placental perfusate in two separate instances: (*a*) endogenously, when the initial control pressor response to bradykinin was observed to be significantly higher than those following, and (*b*) during the bradykinin response that followed the administration of exogenous norepinephrine. Additionally, the pressor response observed with bradykinin was augmented by the prior administration of norepinephrine. Tritium-labeled norepinephrine was administered to the placenta in an attempt to understand better the type of storage taking place, but characterization of the uptake was not possible. The pressor response that bradykinin exerted upon the placenta was not diminished by the preadministration of the parasympatholytic, atropine, or the antiserotonin and antihistaminic drug, diphenhydramine. Administration of α-adrenergic blocking agents to the placenta did, on occasion, reduce the pressor response to bradykinin. However, the evaluation suggests that there is no statistical difference between the pressor response to bradykinin before the administration of phenoxybenzamine, tolazoline, or phentolamine and those responses to bradykinin after their administration.

Keyphrases □ Placenta perfusates—norepinephrine release by bradykinin □ Norepinephrine—release by bradykinin in perfused human placenta □ Bradykinin—pressor-induced release of norepinephrine in perfused human placenta

The actions of bradykinin on various animal tissues and systems have been well detailed in recent years, especially since its isolation, the elucidation of its structure, and synthesis (1, 2). Among the actions of bradykinin are pain production on an exposed blister base (3), increased capillary permeability (4), leukocyte migration (5), an action on both multiunit and visceral smooth muscle (6), and, in conjunction with its effect on vascular smooth muscle, functional vasodilation in relation to secretory glands (7). Furthermore, it appears that this nonapeptide is involved in various shock reactions (8).

In addition to the above-mentioned effects, it has been found that injection of bradykinin into the adrenal artery causes the liberation of catecholamines in animals (9, 10), thereby demonstrating that an autocoid causes the release of a neurotransmitter. Indirect evidence shows a similar type of interrelationship in the rat gut where the inhibitory effect of bradykinin on the intestinal muscle is blocked by the preadministration of the β-adrenergic blocking agent, pronethalol (11). Until the advent of these reports, it was thought that bradykinin exerted its action through a purely musculotropic effect (12), but the literature now suggests an adrenergic component to its action (9–11).

Several investigations (13–15) determined that the placenta and the proximal portions of the umbilical cord are nerve free, leaving the placenta devoid of neural regulation in the performance of its functions. It was also claimed that the placenta is free of catecholamines (16, 17), but other investigations detected their presence in both amniotic fluid and whole placental tissue (18, 19).

This organ has been found to be highly responsive to various agents that contract and relax its musculature (20–22). Agents capable of exerting α- and β-adrenergic receptor stimulation in other tissues also elicit a profound effect on the vasculature of the placenta, demonstrating the presence of specific receptors (23, 24). It was found in this laboratory (25, 26) that the pressor effects of epinephrine and serotonin can be reduced or inhibited by various α-adrenergic blocking agents. Additionally, phentolamine was capable of causing a reversal of the pressor action of epinephrine and, by combination of the α- and β-adrenergic blocking agents, *i.e.*, phentolamine and dichloroisoproterenol, the diphasic action of epinephrine was markedly inhibited.

Several facts and observations, therefore, stimulated interest in this project. Bradykinin was shown (23, 27) to exert a pressor action on the perfused human placenta, although no specific mention as to its mode of action was delineated. The placenta is an ideal organ for the characterization of pharmacologic actions such as musculotropic, direct adrenergic receptor stimulation, or the possible release of some adrenergic mediator substance. Therefore, the purposes of this investigation were to: (*a*) gain more fundamental knowledge of the action of bradykinin in human placental vessels, (*b*) determine any effective antagonists to its action, (*c*) define an adrenergic component of bradykinin's action, be it direct receptor stimulation or mediator release, (*d*) identify the mediator and, finally, (*e*) if a mediator was identified, attempt to discern whether it accumulates in storage sites.

MATERIALS AND METHODS

Placental Perfusion Technique—Full-term human placentas were used in these procedures and were obtained from the hospital 5–15 min. after normal delivery. They were transported to the laboratory in a light-resistant glass vessel containing Tyrode's solution preheated to 38°. The placenta was then placed in a tray containing 38° Tyrode's solution and freed of attached membranes, the umbilical cord was shortened to approximately 3–4 cm., and a warmed 2.3% sodium citrate solution was perfused through one umbilical artery to flush the placenta free of blood.

The additional procedures used in the perfusion experiments in recording and maintaining the perfusion pressure and measuring the inflow and outflow volumes of the perfusate were described in previous papers (28, 29).

Bradykinin triacetate[1] was the principal agonist drug. The additional drugs used in the perfusion experiments were: diphenhydramine hydrochloride[2], 1%; atropine sulfate[3], 0.1%; tolazoline hydro-

[1] Nutritional Biochemical Corp., Cleveland, Ohio.
[2] Benadryl, Parke, Davis & Co., Detroit, Mich.
[3] Mallinckrodt Chemical Works, St. Louis, Mo.

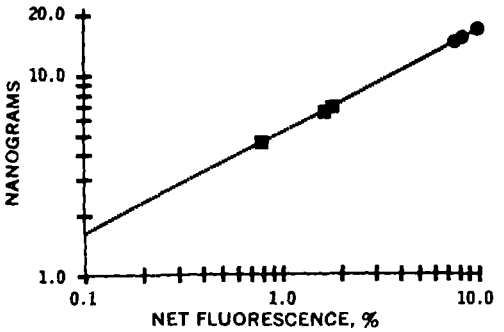

Figure 1—*Key: ..., standard working curve developed for norepinephrine in triple-distilled water; ■, values obtained from the release of endogenous norepinephrine in response to an initial bradykinin injection in the perfused human placenta; and ●, values obtained from the release of administered norepinephrine in response to a subsequent dose of bradykinin in the perfused human placenta.*

chloride[4], 1.0%; phenoxybenzamine[5], 0.2% in ethanol; phentolamine mesylate[6], 0.3%; tyramine hydrochloride[7], 1.0%; and *l*-norepinephrine bitartrate[1], 0.2% (0.1% base). All drugs were injected into a rubber tubing prior to entering the pump *via* a 1.0-ml. glass syringe.

After the placental perfusion system had equilibrated, the usual procedure was to administer two or three doses of bradykinin at predetermined intervals to establish a baseline response. Subsequently, a dose of suspected antagonist would be administered prior to the injection of another dose of bradykinin. In this way, it was possible to observe an increase or decrease in pressor response that could be analyzed statistically. Doses of possible antagonists of bradykinin were obtained by previous investigations in this laboratory (25, 26, 28, 30).

Data on possible antagonism or augmentation were treated statistically by means of a paired Student *t* test. If the data were not paired, a Student *t* test was used (31). A value less than 0.050 was considered significant.

Chemical Analysis of Perfusate—A spectrophotofluorometer[8] was used in these determinations, and results were read directly from the photomultiplier meter. Chemicals were of reagent quality[9] and made fresh each day. All water used was triple distilled, with one distillation being performed from potassium permanganate. The water so prepared was of both low electrical conductivity and organic content.

The ion-exchange procedure was adapted from Bertler *et al.* (32), as suggested by Haggendal (33). However, the resin used was Biorad AG 50 W-X4[10]. It was pretreated[11] in 10-g. quantities rather than milligram amounts to reduce the time required for each subsequent norepinephrine determination.

The norepinephrine used in this determination was stored in a vacuum desiccator and assayed *via* TLC (phenol–water partition system) throughout the procedure to ensure purity. A standard working curve (Fig. 1, dotted line) was developed, using known quantities of this norepinephrine. The curve was developed at the 4-, 6-, 8-, and 10-ng. levels, with three determinations at each concentration. The term "net fluorescence" indicates the number of fluorescent units remaining after subtraction of the reagent blank fluorescent reading from the total fluorescence of the sample.

[4] Priscoline, Ciba Pharmaceutical Co., Summit, N. J.
[5] Dibenzyline, supplied through the courtesy of Smith Kline & French Laboratories, Philadelphia, Pa.
[6] Regitine, supplied through the courtesy of Ciba Pharmaceutical Co., Summit, N. J.
[7] Eastman Organic Chemicals, Rochester, N. Y.
[8] Aminco-Bowman.
[9] HCl, $CuCl_2 \cdot 2H_2O$, $K_3Fe(CN)_6$, NaOH, and Na_2SO_3 (anhydrous), Baker Chemical, Phillipsburg, N. J. KH_2PO_4 and $K_2CO_3 \cdot 1.5H_2O$, Fisher Scientific Co., Fair Lawn, N. J. British Anti-Lewisite, Nutritional Biochemical Corp., Cleveland, Ohio.
[10] Distributed by Calbiochem, Los Angeles, Calif.
[11] (*a*) 1500 ml. 2 *N* NaOH containing 1% ethylenediaminetetraacetic acid, (*b*) 4000 ml. triple-distilled water to remove the NaOH, (*c*) 2000 ml. 2 *N* HCl, (*d*) 4000 ml. triple-distilled water to remove the HCl, (*e*) 1500 ml. 0.1 *M* phosphate buffer, pH 6.5, with 0.1% ethylenediaminetetraacetic acid, and (*f*) 500 ml. triple-distilled water with 0.1% ethylenediaminetetraacetic acid.

Table I—Effect of 10 mg. of Diphenhydramine or of 1 mg. of Atropine on the Action of 50 mcg. of Bradykinin in the Placental Vasculature[a]

Bradykinin Pressor Response before Diphenhydramine, mm. Hg	Bradykinin Pressor Response after Diphenhydramine, mm. Hg	Bradykinin Pressor Response before Atropine, mm. Hg	Bradykinin Pressor Response after Atropine, mm. Hg
6.8	5.6	19.6	14.6
13.2	13.8	15.0	20.0
10.8	11.6	17.0	17.8
		8.6	8.4
$\bar{d} = -0.067$	$t = 0.105$	$\bar{d} = -0.150$	$t = 0.073$
$SE = \pm 0.64$	$p = >0.45$	$SE = \pm 2.05$	$p = >0.45$

[a] *SE* = standard error of the difference. Data paired (*p* greater than 0.050 is not significant).

Radioautographic Technique—DL-Norepinephrine-7-^{3}H was found by the manufacturer's[12] tests to be greater than 98% pure with less than 1% contamination in two separate radiochromatograms: *n*-butanol–acetic acid–water (60:15:25 v/v/v) ascending and 0.2 *M* ammonium acetate–isopropanol (2:1 v/v) ascending, run on cellulose phosphate paper, Whatman No. P81. Two different TLC procedures were performed in this laboratory for purity: phenol–water partition system and *n*-butanol–acetic acid–water (60:15:25 v/v/v). Neither of these systems could detect degradation.

After stabilization of the vascular resistance of the placenta, a pressor response was observed when the tritium-labeled compound was infused (96 mcg.) and the placenta was allowed to continue to perfuse for 5 min. Subsequently, a 5% potassium dichromate solution was perfused through the tissue to complex the norepinephrine and prevent it from washing out with future manipulations in the preparation of histologic sections. Two small portions were then dissected free and prepared by the method of Hempel (34). The specific method selected was the immersion of the tissue for 24 hr. at 4° in 5% potassium dichromate, followed by 72 hr. in Orth's fluid[13] at 4°. The tissue was subsequently superfused with triple-distilled water at 14° for 12 hr. It was embedded for histologic sectioning and mounted onto slides. The prepared slides were dipped into a photographic emulsion[14], placed in a lighttight box for exposure at 3, 7, and 10 days, and developed photographically. At the end of each time interval, they were stained with hematoxylin and eosin for better visualization of the tissue areas.

An evaluation of the location of the exposed silver halide granules was performed by a Student *t* test (31). Exposed granules were classified either as over or touching a cell in the chorionic villus or as not associated with a cell. Counts were obtained from three different tissue areas on three separate slides.

RESULTS

The following results were obtained from full-term human placentas at an initial pressure range of 50–86 mm. (average 71.5) of mercury at a rate of inflow of perfusion fluid of 38–79 ml./min. (average 60.4). The dose of bradykinin selected was 50 mcg.; this amount was sufficient to cause a consistent pressor response but not a maximal response. Several different time intervals were also tried; the results with the 10-min. interval seemed to indicate a decreased responsiveness with repeated administrations. However, a 15-min. interval showed no decrease in responsiveness and was the time selected.

Potential Antagonists—*Anticholinergic and Antihistaminic*—In a total of five placentas, it was observed that neither diphenhydramine (10 mg.) nor atropine (1 mg.) was capable of changing the pressor response exerted in the perfused human placenta by bradykinin (Table I).

[12] Lot 474-223, specific activity 8.76 c./m*M*, New England Nuclear Corp., Boston, Mass.
[13] Orth's fluid: formaldehyde, 10 ml.; potassium dichromate, 2.5 g.; sodium sulfite, 1.0 g.; and water, 100 ml.
[14] Emulsion type-NTB (Nuclear Track Beta), Kodak, Rochester, N. Y.

Table II—Effect of 2 mg. of Phenoxybenzamine on the Action of 50 mcg. of Bradykinin in the Placental Vasculature[a]

Bradykinin Pressor Response before Phenoxybenzamine, mm. Hg	Bradykinin Pressor Response after Phenoxybenzamine, mm. Hg
110.3	90.4
94.0	79.6
6.4	8.4
8.4	13.4
97.6	71.0
15.0	16.4
16.4	14.8
$\bar{d}$ = +7.729	
SE = ±4.73	
t = 1.649	
p = >0.050	

[a] SE = standard error of the difference. Data paired (p greater than 0.050 is not significant).

α-Adrenergic Receptor Blocking Agents—Phenoxybenzamine (2 mg.), on occasion, caused an inhibition of the pressor response of bradykinin. However, in a total of seven experiments performed upon four placentas, no consistent antagonism of the bradykinin responses could be demonstrated (Table II). Similar results were obtained with phentolamine (3 mg.); in 11 experiments on six placentas, no significant difference in the bradykinin response could be demonstrated (Table III). The ineffectiveness of tolazoline in inhibiting the response of bradykinin is shown in Table IV.

Tyramine was then administered to the placenta, with the expectation that it would deplete any norepinephrine present. The initial experiment performed was the infusion of three doses of tyramine (3 mg.) at 5-min. intervals (Fig. 2*a*). Only a small pressor response was observed, the first being slightly higher than the two following. Then a 500-mcg. amount of norepinephrine was infused and, after the α- and β-adrenergic receptor stimulation wore off, another dose of tyramine was given. An increased response was evident, and subsequent doses of tyramine returned the response to the control level.

The same experiment was performed with bradykinin (Fig. 2*b*). Three doses of the peptide were administered, followed by two doses of norepinephrine. A fourth dose of bradykinin was administered, which resulted in a greatly increased response, while a final dose of bradykinin returned the response to normal.

The results of subsequent determinations on the interrelationship of bradykinin and norepinephrine can be observed in Table V. Paired t tests were then performed between the various columns, as outlined in Table VI; a p value of less than 0.050 was considered significant (31). As can be seen in Table VI, the only significantly different bradykinin responses were in six experiments where the initial bradykinin response was higher than the other controls and those responses to bradykinin after loading with norepinephrine.

Table III—Effect of 3 mg. of Phentolamine on the Action of 50 mcg. of Bradykinin in the Placental Vasculature[a]

Bradykinin Pressor Response before Phentolamine, mm. Hg	Bradykinin Pressor Response after Phentolamine, mm. Hg
44.8	63.6
63.6	42.8
16.6	14.4
14.4	11.0
23.0	29.2
29.2	29.0
19.4	27.8
27.8	21.6
10.2	13.2
7.4	12.0
12.0	12.0
$\bar{d}$ = −0.746	
SE = ±2.97	
t = 0.250	
p = >0.4	

[a] SE = standard error of the difference. Data paired (p greater than 0.050 is not significant).

Table IV—Effect of 10 mg. of Tolazoline on the Action of 50 mcg. of Bradykinin in the Placental Vasculature[a]

Bradykinin Pressor Response before Tolazoline, mm. Hg	Bradykinin Pressor Response after Tolazoline, mm. Hg
27.4	26.2
11.0	12.0
42.0	56.4
7.8	6.4
6.4	8.0
10.0	10.6
$\bar{d}$ = −2.50	
SE = ±2.43	
t = 1.029	
p = >0.15	

[a] SE = standard error of the difference. Data paired (p greater than 0.050 is not significant).

Chemical Determinations—Collection of 25-ml. aliquots of effluent was then made as outlined in Fig. 3, and the solution was concentrated by passing it through a microexchange column. Analytical determinations were as follows, as numbered in Fig. 3.

1. All effluent from the initial bradykinin administration was collected. If subsequent administration of bradykinin showed that the initial response was higher than the second, the first effluent was analyzed fluorometrically. Three such observations were made and chemically analyzed; the amounts quantitatively determined were 4.7, 6.7, and 7.1 ng. (Fig. 1).
2. Effluent collection at position 2 of the idealized kymogram and their chemical analysis indicated no detectable amount of norepinephrine from three separate experiments.
3. After loading the placentas with 500 mcg. of norepinephrine, but prior to the injection of bradykinin, the effluent was collected and analyzed. No detectable amount of norepinephrine could be determined in three experiments.
4. The trihydroxyindole derivative of norepinephrine was detectable after administration of bradykinin in position 4 of the idealized kymogram. In three separate determinations, 14.5, 15.25, and 16.75 ng. were observed (Fig. 1).

The presence of bradykinin was determined not to interfere with the analysis, and separate control experiments were run to determine

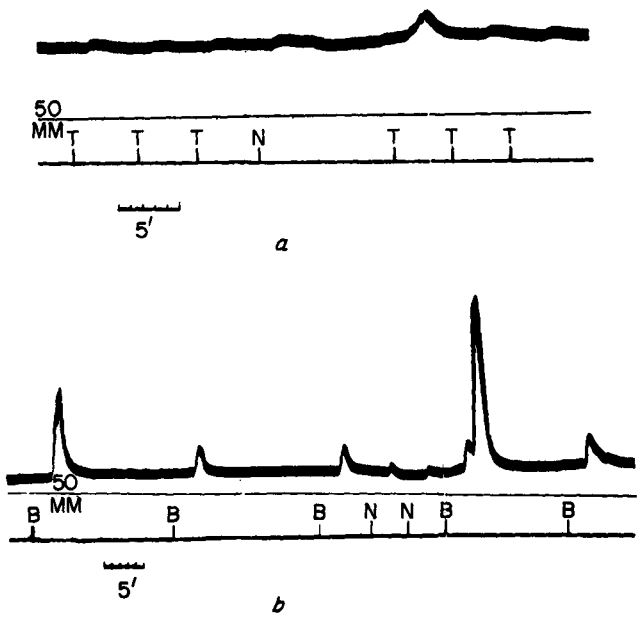

Figure 2—(a) *Response of the perfused human placenta to 3-mg. doses of tyramine as modified by the addition of 500 mcg. norepinephrine.* (b) *Response of the perfused human placenta to 50-mcg. doses of bradykinin as modified by the addition of two 500-mcg. doses of norepinephrine. Key: T = tyramine, 3 mg.; N, = norepinephrine, 500 mcg.; and B = bradykinin, 50 mcg.*

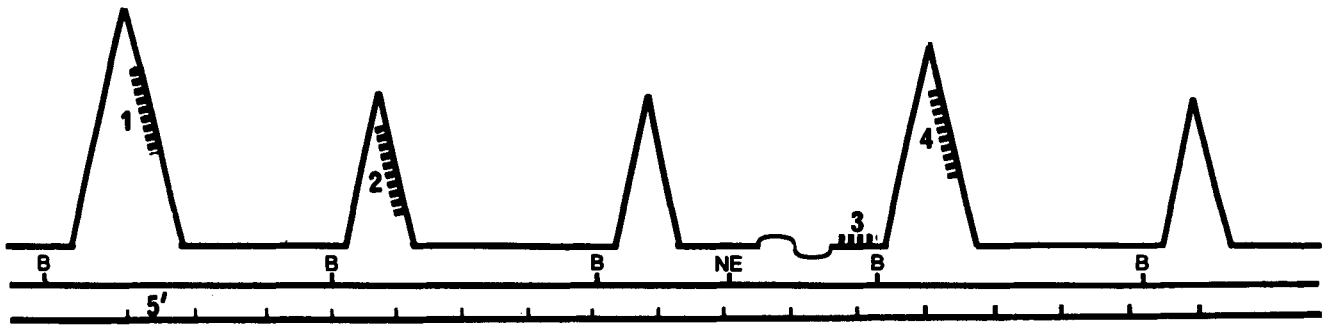

Figure 3—*Idealized kymogram of perfusion regimen and points of perfusate collection for chemical analysis.*

the recovery of norepinephrine in Tyrode's solutions with polyvinylpyrrolidone. Analyses of such solutions were found to be 83% as efficient as those made in pure water.

Microautoradiographs—No statistical difference could be detected between the exposed silver halide granules located over or near cells as opposed to those not associated with cells.

DISCUSSION

It is well known that the human placenta contains acetylcholine (35) which, potentially, could be released, causing a characteristic pressor response (23, 28) which can be blocked by atropine (23). Consequently, should bradykinin be causing a release of endogenous acetylcholine or directly stimulating cholinergic receptors, it would be possible to detect this ability with the use of atropine. After a control response to bradykinin was established, atropine was administered, followed by another dose of bradykinin. In four separate experiments, no decrease in the pressor response of the polypeptide was observed (Table I). Therefore, it seems likely that acetylcholine release is not involved in the pressor response observed with bradykinin in these vessels.

Serotonin and histamine are also known to exert a pressor response on the human placenta, as observed in this laboratory (28, 36) and others (37). Therefore, the possibility of bradykinin working through a mechanism involving serotonin or histamine was also investigated. Diphenhydramine was selected as a potential antagonist to the bradykinin effect, because it possesses a potent antiserotonin and antihistaminic action (26). Unfortunately, the use of this drug in high dosage produces a negative musculotropic effect itself, which is observed by its causing a depression of the placental perfusion pressure (26). In three experiments where diphenhydramine was administered as a potential antagonist in a 10-mg. dose, no diminution in the pressor response of bradykinin was observed (Table I). Increasing the dose of this suspected blocker would have led to inconclusive results because of its strong negative musculotropic action.

Throughout many investigations on organ systems in several animal species, acetylcholine, serotonin, histamine, or their receptors has not been implicated in the mediation of the response of bradykinin and no data in this investigation would lead one to conclude that they are involved. However, suggestions of an adrenergic component in its mechanism of action have arisen. Because the presence of both α- and β-adrenergic receptors was already demonstrated in this laboratory (25, 29), it was hypothesized that the administration of α-adrenergic blocking agents would aid in detecting an adrenergic component that works through bradykinin, directly stimulating α-receptors or releasing endogenous catecholamine. By the use of an α-adrenergic blocking agent, if an adrenergic mechanism were involved in the pressor action of bradykinin, the normal response to the peptide would be diminished.

The results of such attempts may be seen in Tables III–V. While the statistical analysis on each of the three agents selected was negative, several separate experiments demonstrated an apparent inhibitory action. Therefore, some individual experiments suggest an α-receptor-stimulating component to the pressor action of bradykinin, although it is certainly not always observed.

Several independent observations in this laboratory lead one to the assumption that there might be an endogenous release of norepinephrine (Fig. 2*b*). On occasion, the initial response to a series of two or three doses of bradykinin would be substantially higher than those responses following. This observation could not be attributed to a classical definition of tachyphylaxis, because no further decrease

Table V—Responses of the Placental Vasculature to 50 mcg. of Bradykinin as Modified by the Addition of 500 mcg. of Norepinephrine

Control Placental Response with Bradykinin (1), mm. Hg	Control Placental Response with Bradykinin (2), mm. Hg	Control Placental Response with Bradykinin (3), mm. Hg	Bradykinin Response after Infusion of Norepinephrine (4), mm. Hg	Final Bradykinin Response (5), mm. Hg
14.0	7.2	6.0	9.8	8.0
—	10.0	8.4	12.2	8.0
50.0	16.0	16.0	103.0	17.8
—	16.0	15.8	25.2	16.8
—	39.4	47.0	57.4	—
20.0	7.4	8.6	12.3	9.0
38.4	17.0	9.0	23.8	—
41.6	23.0	20.6	40.4	—
9.0	2.0	2.4	3.2	2.4
—	3.8	3.6	5.0	4.4
—	11.0	9.6	12.6	9.4
—	9.4	9.6	13.0	—
—	3.0	2.8	6.0	4.8
—	4.6	4.6	6.4	—

Table VI—Statistical Evaluation on the Response of the Placental Vasculature to 50 mcg. of Bradykinin as Modified by Addition of 500 mcg. of Norepinephrine

Responses Evaluated[a]	$\bar{d}$[b]	$(n-1)$[c]	SE[d] (±)	t	p
(1) to $\frac{(2)+(3)}{2}$	+17.57	5	4.42	3.972	<0.01[e]
(2) to (3)	+ 0.414	13	0.85	0.485	<0.35
$\frac{(2)+(3)}{2}$ to (4)	−11.67	13	5.97	1.955	<0.05
$\frac{(2)+(3)}{2}$ to (5)	− 0.644	8	0.37	1.759	<0.1

[a] Numbers in parentheses refer to columns in Table V. [b] $\bar{d}$ = mean of difference. [c] Degrees of freedom. [d] SE = standard error of the difference. [e] p values less than 0.050 are significant.

was observed with subsequent doses at the 15-min. time interval. It was considered possible that any suspected catecholamine would be released at once and that only resupplying that amine would allow an increased response to bradykinin to be observed once again.

To test this hypothesis, tyramine was used initially because it is known to deplete norepinephrine and, hence, produce tachyphylaxis. Three doses were administered to the placenta, and a small, consistent pressor response was observed, probably due to a direct positive musculotropic action, direct α-adrenergic receptor stimulation, or catecholamine release (Fig. 2*a*). Subsequently, a dose of norepinephrine was administered and, after the α- and β-adrenergic receptor stimulation wore off, another dose of tyramine was given. The ensuing pressor response observed was markedly higher than the responses of the controls. The last two doses of tyramine returned the response to control levels.

To continue testing the hypothesis, bradykinin was administered in three successive 50-mcg. doses at 15-min. time intervals (Fig. 2*b*), the initial response being markedly higher than the two following responses. Two 500-mcg. doses of norepinephrine were administered, and bradykinin was given again after its α- and β-adrenergic receptor stimulation subsided at the required 15-min. time interval. A dramatic increase in the pressor response was obtained, and the following dose of bradykinin returned the pressor response to the prior control levels (Fig. 2*b*). Evaluation of the results obtained in 14 placentas confirmed this initial observation (Tables V and VI). In all 14 experiments, if the initial bradykinin response was obviously higher than the second response, a third control was done. Table VI demonstrates that there was a statistical difference between the initial pressor responses of six placentas as compared to the average pressor responses obtained in other controls for the same placenta $\left[(1) \text{ to } \frac{(2)+(3)}{2}\right]$; in the remaining placentas and in the second and third controls of the above-mentioned six placentas, no difference could be detected [(2) to (3)].

After loading the placenta with norepinephrine, the subsequent response to bradykinin was also greater $\left[\text{Table VI, } \frac{(2)+(3)}{2} \text{ to (4)}\right]$. The response to a final dose of bradykinin returned to control levels $\left[\text{Table VI, } \frac{(2)+(3)}{2} \text{ to (5)}\right]$. Consequently, there appeared to be a strong implication of catecholamine release from the placenta by bradykinin augmenting the known positive musculotropic pressor action of this peptide.

The chemical determinations were sensitive enough to detect accurately 1.75 ng. or more of norepinephrine in pure solution. The wavelengths selected, 325 nm. for activation and 510 nm. for fluorescence (uncorrected instrument values), were able to discriminate between norepinephrine and other catecholamines and metabolites. In all placental perfusion experiments carried out for chemical analysis, the perfusate from the initial bradykinin pressor response was collected and analyzed (Fig. 3). Norepinephrine was determined in three of the initial effluents, and those three positive analyses corresponded to increased pressor responses observed in comparison to the following bradykinin responses (Figs. 1 and 3). All other analyses of initial bradykinin–pressor response perfusate proved to be negative for norepinephrine. However, at no time was an increased pressor response observed that did not prove to be positive for norepinephrine content.

Norepinephrine was infused into the placenta and, after the α- and β-adrenergic receptor stimulation subsided but before the next dose of bradykinin was administered, perfusate was collected to determine if there was any residual release of the norepinephrine; none could be detected. Bradykinin was then administered again at the proper 15-min. interval and the perfusate was collected. Norepinephrine was found to be present in all three analyses (Figs. 1 and 3). The pressor response was also significantly increased, as shown in Tables V and VI (Fig. 2). Following doses of bradykinin returned the pressor response to the preaugmented response.

Barium chloride, a chemical known to exert its constrictor action upon smooth muscle *via* a positive musculotropic effect, was ineffective in causing the release of preadministered norepinephrine in two experiments, although it caused a potent pressor action in the placenta itself. Therefore, the release of norepinephrine is not the result of a general positive musculotropic action.

Observations on the increased pressor response of bradykinin in conjunction with the consistent presence of norepinephrine leads to two conclusions: (*a*) the human placenta can take up and store norepinephrine, and (*b*) bradykinin can release those stores.

With the use of tritium-labeled norepinephrine, it was hoped that some insight on the nature of the storage would be demonstrated. It was hypothesized that this storage would be either: (*a*) distinct areas restricted to a cellular locale and analogous to the storage that might be visualized in a nerve terminus or (*b*) no less specific but more diffuse in its nature. The experiment was carried out by depleting any prestored catecholamine with the administration of usual doses of bradykinin. Labeled norepinephrine was administered, and the placenta was flushed free of residual radioactivity for 5 min.

The procedure already outlined for the fixation of the water-soluble catecholamines in tissue is qualitatively similar to other methods (34). However, this method is quantitatively more efficient because, rather than retaining only 10% as seen in other methods, it retains 60–80% of the tritiated catecholamines in the tissues.

After exposure of the slides, they were developed photographically and the tissue was stained with hematoxylin and eosin. The slides were evaluated statistically with a Student *t* test to determine whether there was any difference between those exposed silver halide granules that were associated with a cell and those that were not associated with cells. The analysis showed no difference between the two groups. Consequently, there seemed to be no distinct loci for the uptake of the norepinephrine.

As a control for the experiment, the procedure was repeated with nonlabeled norepinephrine and the same protocol was followed to determine if the potassium dichromate or Orth's fluid would chemically expose the photographic emulsion. No such exposed silver halide granules were observed. This portion of the investigation proved inconclusive and an interpretation of the results is difficult. Therefore, no conclusions on the storage of norepinephrine are possible.

The whole question of nonneuronal storage of norepinephrine is unresolved. Several investigations showed that it does take place, but all of these investigations made use of denervated tissue (38, 40), not noninnervated tissue as found in the placenta. With denervation of a tissue, degeneration of the distal nerve structures takes place, including the storage granules of bound norepinephrine. Such data suggest that in the denervated rat salivary gland, extraneuronal stores of norepinephrine are present that are resistant to depletion by chemical means (38, 39). Draskoczy and Trendelenberg (40), using the cat nictitating membrane, measured an increasing amount of radioactivity after exposure to increasing amounts of labeled norepinephrine and suggested that the uptake appears to be extraneuronal but not extracellular. It should be determined if these stores consist of both unbound and bound pools or only of the unbound form. The nature of any extraneuronal pool must be different from those extraneuronal stores observed in the denervated rat salivary gland, because the rat salivary stores are resistant to drug depletion whereas the placental stores are at least partially depletable by bradykinin.

Therefore, with the aforementioned facts in mind, reevaluation of several theories and results obtained in this laboratory and others is in order. The concept of the human placenta being free of catecholamines should be discarded. Furthermore, realization of the fact that the human placenta is capable of storing and releasing norepinephrine suggests a modification on the thinking of mother–placenta–fetus interactions with respect to endogenous autocoids or exogenous drugs which may release or alter their storage and thereby affect vascular responses.

SUMMARY

1. The pressor response bradykinin exerted upon the perfused human placenta was not diminished by the preadministration of the parasympatholytic atropine or the antiserotonin and antihistaminic drug diphenhydramine.

2. Administration of α-adrenergic receptor blocking agents to the placenta did, on occasion, reduce the pressor response to bradykinin. However, evaluation of several experiments suggests that there is no statistical difference between the pressor response to bradykinin before the administration of phenoxybenzamine, tolazoline, or phentolamine and those responses to bradykinin after their administration. The possibility of some direct α-adrenergic receptor stimulation remains.

3. The pressor response observed with bradykinin is augmented by the prior administration of norepinephrine.

4. Norepinephrine was detectable chemically in the placental perfusate in two separate instances: (*a*) when the pressor response to bradykinin was observed to be significantly higher than the other controls, and (*b*) during the bradykinin response following the administration of exogenous norepinephrine.

5. Norepinephrine was not detectable chemically in other control responses to bradykinin or in the effluent collected between the administration of exogenous norepinephrine and a following dose of bradykinin.

6. Release of norepinephrine in this tissue is not the result of a general positive musculotropic action, because effluent analysis of the norepinephrine-primed placenta after administration of barium chloride proved negative.

7. The minimum amount of endogenous norepinephrine detected was in the order of 4 ng. Undoubtedly, the total catecholamine content of the human placenta will be found to be greater than this amount.

8. Tritium-labeled norepinephrine was administered to the perfused human placenta in an attempt to understand better the type of storage taking place. Interpretation of the data is difficult and, therefore, no conclusions as to the type of storage of norepinephrine are possible.

9. Realization that the human placenta can store and release norepinephrine suggests a reevaluation of autonomic drug action on the placenta, both for basic placental pharmacology and maternal–placental–fetal implications.

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