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THE EFFECTS OF ADRENALINE, NORADRENALINE AND DIHYDROERGOTAMINE ON EXCISED HUMAN MYOMETRIUM

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The response of the uterus to adrenal medullary hormones differs among the various species, and may, in some species, differ in the pregnant and non-pregnant states. In an earlier communication it was shown that adrenaline inhibited, and noradrenaline stimulated, the large co-ordinated contractions of the intact, late pregnant and labouring human uterus (Garrett, 1954). In this paper the responses of excised strips of human myometrium to these hormones are described.

Adrenaline *B.P.* (Epinephrine *U.S.P.*) prepared from natural sources is now known to contain 15 to 20% noradrenaline. Therefore, if previous workers reporting on the action of adrenaline have not stated that they used the pure synthetic substance, it follows that some revision of their work is necessary, lest the presence of noradrenaline as an impurity had vitiated their results.

Greef and Holtz (1951a and b) have repeated the experiments of earlier workers with excised tissue *in vitro*, using pure adrenaline and noradrenaline. They have shown that noradrenaline has a consistent excitatory effect during parturition in the rabbit, dog, guinea-pig and rat. Their observations with adrenaline in these animals simply confirm the findings of past workers, whose results were summarized by Gunn (1944).

A few experiments were done with human tissue in earlier years. Rubsamen and Klingerman (1912) (quoted by Gunn and Scott Russell, 1946) examined strips of myometrium from the pregnant uterus at term, and showed that adrenaline caused a rise in tone and an increase in the individual contractions.

Gunn (1914) examined strips from one non-pregnant uterus. He recorded the spontaneous contractions of the myometrium and found that these were stimulated by adrenaline. These few experiments were repeated on a much larger scale by Gunn and Scott Russell (1946), who showed that adrenaline always stimulated the contractions of excised human myometrium from both pregnant and non-pregnant uteri.

The present work is an attempt to amplify previous findings by examining the response of excised human myometrium to the pure substances (−)-adrenaline and (−)-noradrenaline.

METHODS

The apparatus for recording the contractions of exsected human myometrium was of the familiar type used for isolated organs, following the method of Gunn and Scott Russell (1946).

The strip of myometrium was suspended in a 10-ml. cylinder containing Ringer-Locke's solution through which a continuous flow of oxygen was bubbled. The composition of the fluid was: NaCl 9 g., KCl 0.42 g., $CaCl_2$ 0.24 g., $NaHCO_3$ 0.2 g., glucose 0.1 g., distilled water to 1,000 ml. The bath was held in a large water jacket at 37° C., and was fed through a narrow coiled glass tube of 25 ml. capacity, so that the specimen could be twice washed without any change in the temperature of the Ringer-Locke's solution. The strip of myometrium in the bath was fixed at one end to a glass rod, and at the other to a light lever recording on a smoked paper. Tracings of these records were made for reproduction here.

In all, 71 strips from 40 uteri were examined. The strips from non-pregnant uteri obtained at hysterectomy were taken vertically from the midline of the anterior surface of the body of the uterus, and were approximately of uniform size, 30 to 40 mm. × 3 mm. × 3 mm. Those from pregnant uteri obtained at caesarean section were cut transversely from the edge of the incision in the lower segment operation and were of variable size.

Most specimens were examined within a few hours of their removal, but sometimes they were stored in Locke's solution in a temperature-controlled refrigerator, at 10° C., for up to 48 hr. before records were obtained.

As Gunn and Scott Russell found, spontaneous rhythm was not established for a variable time after

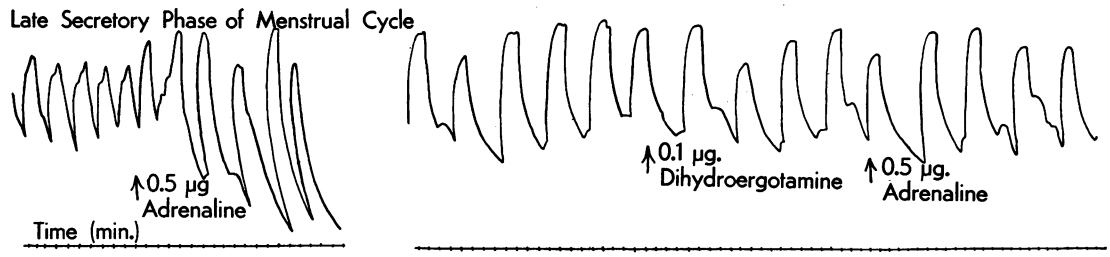

FIG. 1.—Non-pregnant myometrium in the late secretory phase of the menstrual cycle. Each dose on tracing is the final concn./ml. Adrenaline 1: 2,000,000 causes augmentation of the established activity. In the same specimen dihydroergotamine 1: 10,000,000 blocks the response to adrenaline.

the specimens were set up in the apparatus. Rather than initiate contractions by chemical or mechanical stimuli, records were not begun until this latent period had passed and the spontaneous rhythm was well established and constant.

RESULTS

The latent period before spontaneous rhythm was established varied with the physiological state of the material. Post-menopausal myometrium exhibited regular rhythm half to one hour after suspension in the bath; myometrium obtained during menstrual life began to contract in 2 to 3 hr., whereas specimens from late pregnancy and labour frequently failed to show any spontaneous activity at all, if examined within 24 hr. of removal. These pregnant specimens, however, if kept 24 to 48 hr. after their removal at caesarean section, showed contractions 2 to 3 hr. after being set up in the bath.

Failure to wait for this latent period to pass, together with the use of normal saline in place of Ringer-Locke's solution, doubtless explains Goldfarb's (1954) unusual results with pituitary extract on excised myometrium in late pregnancy.

Adrenaline and Noradrenaline

Non-pregnant Uterus

Before the Menopause.—Both adrenaline (as hydrochloride) and noradrenaline (as bitartrate) stimulated the non-pregnant human myometrium *in vitro*. With low concentrations of the active bases (e.g. up to 1 in 2,000,000), augmentation of established activity was observed (Fig. 1), but with higher concentrations (1 in 1,000,000 to 1 in 200,000) the muscle went into spasm, which persisted for some minutes (Fig. 2).

This reaction was obtained with adrenaline in 15 strips from 17 uteri. Doses well below the threshold concentrations produced no reaction in the remaining 2 specimens (Table I).

Stimulation was similarly observed with noradrenaline in strips from 17 out of 19 non-pregnant uteri. Subthreshold concentrations accounted for one of the specimens failing to respond. In the other specimen no reaction occurred with concentrations up to 1 in 200,000; with the excessively high concentration of 1 in 20,000, there was inhibition (Table I).

After the Menopause.—Similar responses to adrenaline and noradrenaline were obtained with the 8 strips examined from 5 post-menopausal uteri (Fig. 3).

A comparison, by the method of Litchfield and Wilcoxon (1949), of the concentrations of adrenaline and noradrenaline producing stimulation in 50% of the specimens (the ED50) showed that non-pregnant myometrium is 2½ times more sensitive to noradrenaline than to adrenaline.

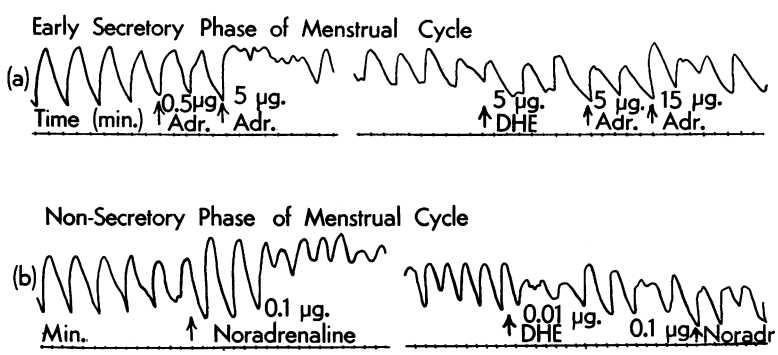

FIG. 2 (*a*) and (*b*).— Non-pregnant myometrium. Both adrenaline and noradrenaline stimulate the contracting myometrium. This response is blocked by dihydroergotamine. Each dose on tracing is the final concn./ml.

For all non-pregnant specimens the ED50 (and 19/20 confidence limits) for adrenaline was 1.38 (0.72 to 2.65) μg./ml., and for noradrenaline was 0.39 (0.21 to 0.71) μg./ml. The potency ratio is, therefore, $\frac{\text{Noradrenaline ED50}}{\text{Adrenaline ED50}}$ (and 19/20 confidence limits) = 2.4 (1.5 to 8.5).

Although differences were observed between the responses of specimens obtained during the endometrial, proliferative, and secretory phases of the menstrual cycle, these apparent variations were not statistically significant.

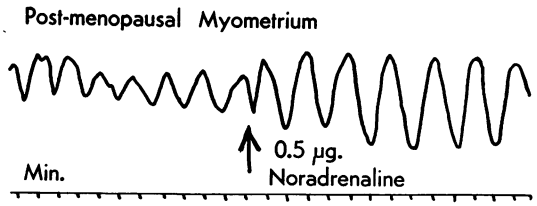

FIG. 3.—Augmentation of the spontaneous contractions of post-menopausal myometrium produced by noradrenaline 1 : 2,000,000. Dose on tracing is the final concn./ml.

Pregnant Uterus

Early Pregnancy.—One specimen from the first trimester was obtained at hysterotomy from a

TABLE I

EFFECTS OF ADRENALINE AND OF NORADRENALINE ON STRIPS FROM INDIVIDUAL NON-PREGNANT UTERI

Phase	Case No.	Adrenaline (μg./ml.)							Noradrenaline (μg./ml.)							
		0·001	0·01	0·1	0·5	1	5	20	0·001	0·01	0·1	0·5	1	5	20	50
Proliferative ..	1			0							0					
	2				0	0	+						0	+		
	3										+	+		+		
	4			0	0											
	5										0	0		+		
	6			0	0		+				0	+		+		
	7				0		0	+				0			+	
	8											0	0	±	+	
	9					0	+				+			+		
	10				0		+				+		+	+		
	11										0		0	0		−
	12													+		
Secretory ..	13	0	0	0	+				0	0	0	+	+			
	14				0	0	+	+								
	15					+							+			
	16		0	0	+	+					0	+				
	17				0	0	+									
	18				0	0	0	+				0		+		
	19						+							+		
	20				+						+					
	21				0		+					+				
	22				+								0		+	
Post-menopausal	23				0	0	+					+				
	24						+							+		
	25		0	0	0		+				+	+	+			
	26						+							+		
	27				0		0					0		+		

0 = No effect. + = Stimulation. ± = Equivocal response. − = Inhibition.

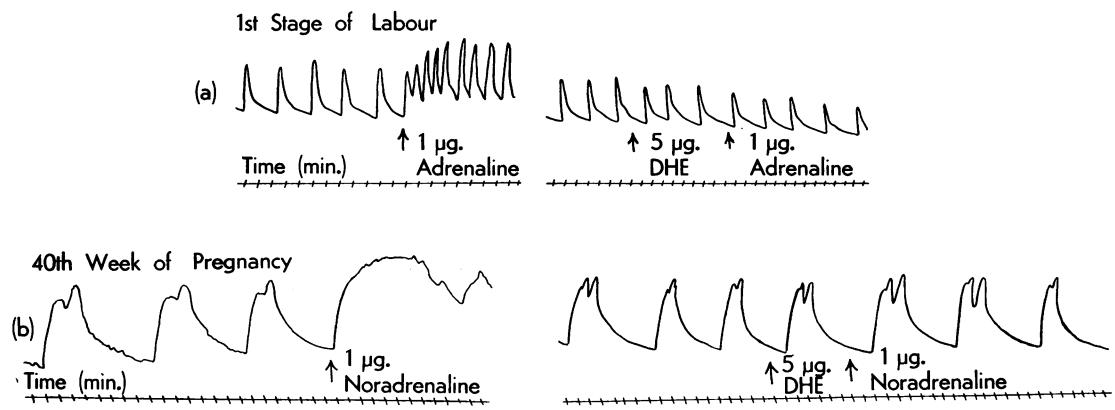

FIG. 4.—Pregnant myometrium from the lower uterine segment. (*a*) At 1st stage of labour, and (*b*) at 40th week of pregnancy. Each dose on tracing is the final concn./ml. Both adrenaline and noradrenaline stimulate the contracting myometrium. This response is blocked by dihydroergotamine.

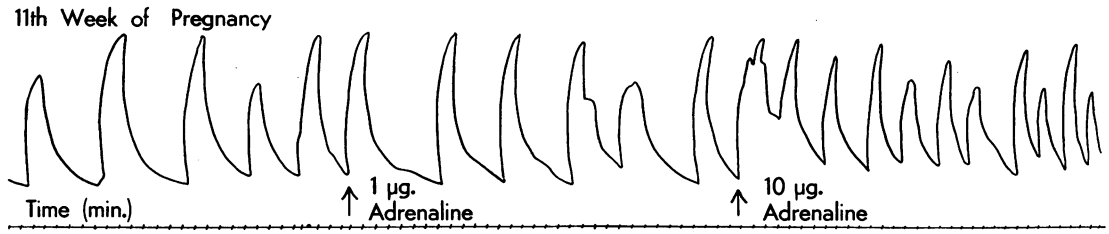

FIG. 5.—Myometrium from the body of the uterus in the first trimester. Each dose on tracing is the final concn./ml. Adrenaline produces an equivocal result: in concentration of 1: 100,000 there is increased frequency but decreased amplitude of contractions with slight rise in base line.

patient who gave a history of 11 weeks' amenorrhoea. Myometrial strips from this case were stimulated by noradrenaline, but gave equivocal results with adrenaline. The latter caused increased frequency of contraction with some raising of the base line, but with decreased amplitude (Fig. 5).

Late Pregnancy and Labour.—In the third trimester both adrenaline and noradrenaline stimulated the myometrium to contract. This reaction was obtained in strips from 9 out of 10 uteri with adrenaline, and with strips from all 10 uteri with noradrenaline (Table II).

These results with pregnant specimens from the lower uterine segment cannot be quantitatively compared with the other specimens in the whole series, as the non-pregnant specimens came from the body of the uterus. Gunn and Scott Russell (1946) showed that muscle from the upper segment is less sensitive to adrenaline than that from the lower segment, although their responses are qualitatively similar.

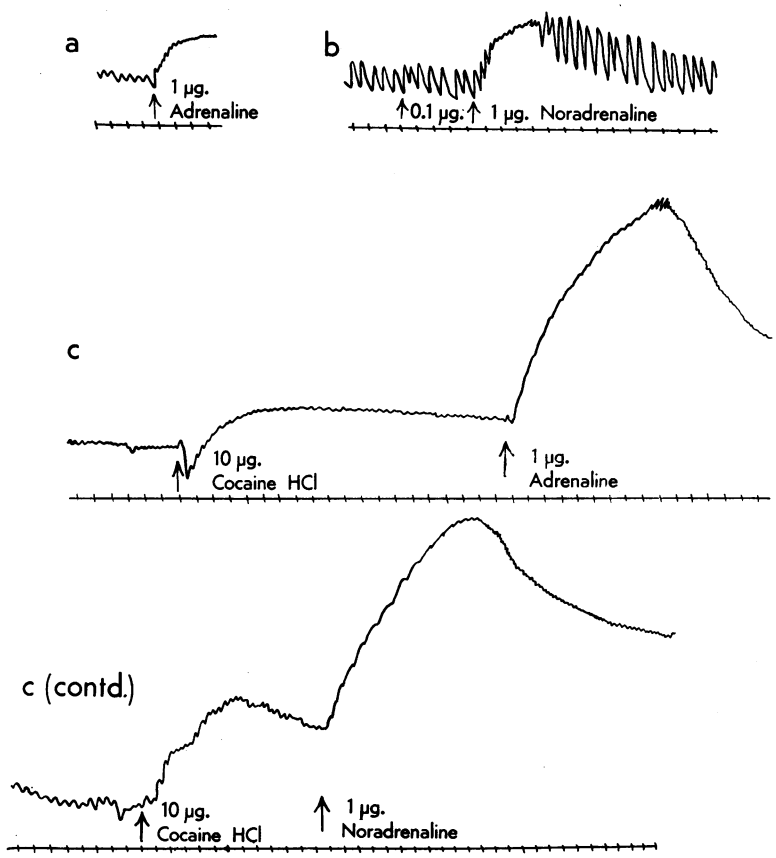

FIG. 6.—Three strips of myometrium from the same uterus at the 39th week of gestation. Each dose on tracing is the final concn./ml. In *a* and *b* adrenaline and noradrenaline produce stimulation. In *c* stimulation is also seen with these drugs in the presence of cocaine HCl 1: 100,000. Time in min.

The usual stimulation observed with adrenaline and noradrenaline in strips from both pregnant and non-pregnant uteri persisted after the addition of cocaine hydrochloride to the bath (Fig. 6).

Dihydroergotamine

Dihydroergotamine itself has usually no direct action on spontaneously contracting myometrium *in vitro*. Occasionally it has inhibited contractions in non-pregnant specimens and stimulated contractions in a few pregnant specimens, but such responses are the exception, not the rule.

In 7 out of 10 non-pregnant uteri, and in all 6 pregnant uteri examined, the usual stimulating action of adrenaline was not seen after the addition of dihydroergotamine to the bath (Figs. 1, 2a, and 4a). In fact, its action was occasionally

TABLE II

EFFECTS OF ADRENALINE AND OF NORADRENALINE ON STRIPS FROM INDIVIDUAL PREGNANT UTERI

Phase	Case No.	Adrenaline (μg./ml.)					Noradrenaline (μg./ml.)			
		0·1	0·5	1	5	10	0·1	0·5	1	5
Early pregnancy	28			0		±		+	+	+
Late pregnancy and labour	29				+		0	0		+
	30	0	0	+			0	0		+
	31	+								
	32		0		0	0		+	+	
	33		0		+			0		+
	34				+					+
	35									+
	36				+					+
	37	+		+			0		+	
	38			0		+			+	
	39									+
	40			+						

0=No effect. +=Stimulation. ±=Equivocal response.

TABLE III

DETAILS OF EXPERIMENTS DEMONSTRATING THE ADRENALINE-BLOCKING PROPERTIES OF DIHYDRO-ERGOTAMINE

Phase	Case No.	Stimulating Concn. of Adrenaline/ (μg./ml.)	Concn. of DHE (μg./ml.)	Repeated Concn. of Adrenaline (μg./ml.)	Ratio: Repeated Concn. Adrenaline/ Concn. DHE	Result*
Non-pregnant	22	0·5	0·5	0·5	1	—
	9a	10	10	10	1	0
	9b	10	20	20	1	—
	21a	5	5	5	1	0
	16a	1	0·5	1	2	0
	14	20	10	20	2	+
	18	20	5	20	4	—
	21b	5	5	20	4	0
	20	0·5	0·1	0·5	5	0
	16b	1	0·5	5	10	0
	6a	5	0·5	5	10	0
	6b	5	0·5	10	20	0
	25	5	0·1	5	50	+
	23	5	0·1	5	500	+
Pregnant	40b	1	5	1	0·2	0
	36	5	10	5	0·5	0
	31	0·1	0·1	0·1	1	0
	40a	1	1	1	1	+(much reduced)
	33	5	5	5	1	0
	34a	5	5	5	1	0
	34b	5	5	20	4	—
	30a	1	0·1	1	10	0
	30b	1	0·1	5	50	0

* 0=No effect. +=Stimulation. −=Inhibition.

reversed, and definite inhibition was observed (Table III).

Similarly, dihydroergotamine blocked the stimulating action of noradrenaline in 8 out of 11 non-pregnant uteri and in all 6 pregnant uteri examined (Figs. 2b and 4b; Table IV).

DISCUSSION

It has been shown that the spontaneous contractions of isolated human myometrium are stimulated by both adrenaline and noradrenaline, and that this reaction is qualitatively the same whether the specimen is obtained during one of the various stages of the menstrual cycle, after the menopause, during pregnancy or at parturition.

The site of action of adrenaline and noradrenaline is probably on the plain muscle cells themselves rather than on the nervous elements included in the myometrial strip. When the nerve endings are paralysed by cocaine the responses to adrenaline and to noradrenaline persist.

From the point of view of comparative physiology, the human uterus must then be placed in the "rabbit group" of Greef and Holtz's table; this includes the animals in which adrenaline and noradrenaline stimulate both the pregnant and non-pregnant uterus. As all of this table is based on evidence from studies *in vitro*, this is justifiable, but there are certain other factors which will now be considered.

Kaiser (1951) showed, and Garrett (1954) confirmed more directly, that adrenaline *inhibits* the

TABLE IV

DETAILS OF EXPERIMENTS DEMONSTRATING THE NORADRENALINE-BLOCKING PROPERTIES OF DIHYDRO-ERGOTAMINE

Phase	Case No.	Stimulating Concn. of Noradrenaline (μg./ml.)	Concn. of DHE (μg./ml.)	Repeated Concn. of Noradrenaline (μg./ml.)	Ratio: Repeated Concn. Noradrenaline/ Concn. DHE	Result*
Non-pregnant	21a	0·5	1	0·5	0·5	0
	27	5	10	5	0·5	0
	20a	0·1	0·1	0·1	1	0
	16a	0·5	0·5	0·5	1	0
	9a	2	2	2	1	0
	18	5	5	5	1	0
	16b	0·5	0·5	1	2	0
	21b	0·5	1	2	2	0
	20b	0·1	0·1	0·5	5	—
	21c	0·5	1	5	5	0
	9b	2	2	10	5	0
	3a	0·1	0·01	0·1	10	0
	25a	0·1	0·01	0·1	10	+
	6a	5	0·5	5	10	+(reduced)
	6b	5	0·5	15	30	+
	3b	0·1	0·01	0·5	50	0
	23	0·5	0·01	0·5	50	+
	25b	0·5	0·1	5	50	+
	5	5	0·1	5	50	0
Pregnant	37b	1	25	1	0·04	0
	37a	1	10	1	0·1	+(reduced)
	37c	1	25	5	0·2	+(much reduced)
	38	1	5	1	0·2	0
	35b	5	20	5	0·25	0
	36	5	10	5	0·5	0
	35a	5	5	5	1	+(much reduced)
	33a	5	5	5	1	0
	33b	5	5	15	3	0
	31	5	0·1	5	50	0

* 0=No effect. +=Stimulation. −=Inhibition.

contractions of the intact human uterus in late pregnancy and labour, whereas noradrenaline stimulates them. As far as adrenaline is concerned this would appear to be a direct conflict of results, yet each of these conclusions has been drawn from experiments in which the results have always proved consistent. It may be said that *in vitro* methods are so unnatural as to produce spurious responses, but the results have invariably been the same. If they are to be accepted as being indicative of the physiological processes involved in uterine contraction, we are faced with a seeming conundrum. Perhaps the contractions measured *in vitro* are a different entity altogether from the large co-ordinated contractions of the whole uterus observed in the patient in late pregnancy and parturition. If so, further work is necessary to produce a satisfactory synthesis of the results of experiments to date.

Just as Orth and Ritchie (1947) have shown in the heart, and Greef and Holtz (1951a) have shown in the uterus of lower mammals, that dihydroergotamine can block the characteristic response to adrenaline and noradrenaline, so too in this present work has this been shown to be true of excised human uterine muscle. However, these adrenaline-blocking properties have yet to be demonstrated *in vivo*; the writer is studying this problem at present.

SUMMARY

1. Experiments *in vitro* with strips of myometrium from 40 human uteri are described.

2. Both adrenaline and noradrenaline stimulate the spontaneous contractions of human myometrium obtained at the various stages of the menstrual cycle, after the menopause, during pregnancy and at parturition.

3. Noradrenaline has a greater stimulant action on non-pregnant myometrium than has adrenaline.

4. The stimulant action of adrenaline and noradrenaline on human myometrium persists in the presence of cocaine.

5. Dihydroergotamine blocks the stimulant action of adrenaline and noradrenaline on human myometrium *in vitro*.

6. These *in vitro* results are compared with the known actions of adrenaline and noradrenaline on the intact human uterus in late pregnancy and parturition. The *in vitro* and *in vivo* results differ in certain essential respects.

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