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Oestrogen-induced sensitization of the uterine artery of the guinea-pig to acetylcholine

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Summary

1. It has been shown previously that a dilatation of the uterine artery of the guinea-pig in response to acetylcholine or to stimulation of cholinergic vasodilator nerves occurs only during pregnancy. Experiments have now been performed to determine the hormonal basis of this change in responsiveness.

2. Chronic treatment of virgin guinea-pigs with oestradiol, either by intramuscular injection or by subcutaneous implantation, caused the development of a responsiveness of the uterine artery to acetylcholine which was similar in degree to that occurring during pregnancy.

3. Simultaneous administration of progesterone did not significantly inhibit this oestrogen effect. In contrast the characteristic actions of oestrogen on uterine growth and fat catabolism were completely antagonized by progesterone.

4. The time course of the development of responsiveness to acetylcholine of the uterine arteries during pregnancy was parallel to the rises in oestrogen production and placental blood flow as determined by other workers. This supports the concept that cholinergic dilator nerves participate in uterine hyperaemia during pregnancy and that their functioning is regulated by the maternal blood oestrogen level.

Introduction

The main parametrial artery of the guinea-pig receives a population of cholinergic vasomotor nerves. During the latter half of pregnancy, exogenous acetylcholine or stimulation of these nerves cause vasodilatation, but at other times both stimuli have no appreciable effect. This unusual situation has led to the suggestion that cholinergic vasodilator nerves may be involved in the production or maintenance of uterine hyperaemia during pregnancy (Bell, 1968, 1969). The present paper describes experiments which indicate that the onset of the sensitivity of the artery to acetylcholine during pregnancy is linked to the rise in blood oestrogen concentrations. Some of the results were reported to the British Pharmacological Society in March, 1970 (Bell, 1970).

Methods

Female guinea-pigs were killed by cervical dislocation and the parametrial arterial supply was prepared for use as an isolated, perfused preparation, as described previously (Bell, 1968). Constrictor responses to submaximal (Bell, 1968) intra-

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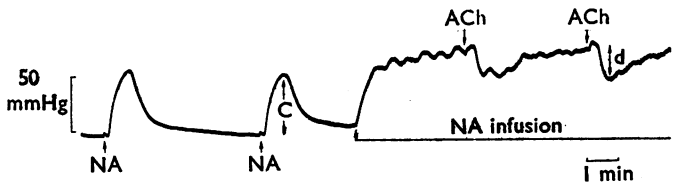

FIG. 1. Perfusion pressure record of an isolated, perfused uterine artery taken from a virgin guinea-pig which had been treated for three days preceding the experiment with oestradiol-17β valerate 1 mg/day i.m., showing the method by which vessel sensitivity to acetylcholine was assessed. At NA, noradrenaline 0·5 μg was injected intraluminally, following which the tone of the vessel was kept elevated by an infusion of noradrenaline (0·5 μg/ml). Under these conditions acetylcholine (ACh) 50 ng was injected intraluminally. Vessel responsiveness to acetylcholine was expressed as the ratio between the amplitudes of responses to NA and ACh, calculated as a percentage ($\frac{d}{C} \times 100$).

luminal doses of noradrenaline (0·5 μg) were recorded. Subsequently the tone of the preparation was kept elevated by a continuous intraluminal infusion of noradrenaline (0·5 μg/ml) during which the dilator responses to submaximal doses of acetylcholine (50 ng) were tested. The sensitivity of the artery to acetylcholine was derived from the ratio between the amplitudes of the responses to acetylcholine and to noradrenaline, calculated as a percentage (Figure 1). Sometimes the arteries responded to the first dose of acetylcholine (Bell, 1968), but remained subsequently insensitive. In such cases the first response was disregarded.

In pregnant animals the day on which a vaginal plug was found was taken as day 1. Gestational age was further checked by comparing the lengths of the foetuses with the data recorded by Draper (1920). All pregnant animals were primiparous.

All non-pregnant animals used were virgins weighing 350–500 g. The day of oestrus was taken as that day on which the vaginal membrane was found ruptured; all other untreated animals were regarded as being in dioestrus. Two types of oestrogen treatments were employed: (1) intramuscular injection with an oil-based preparation of either oestradiol benzoate (Oestroform; B.D.H.) 0·5 mg/day or oestradiol valerate (Primogyn; Schering) 0·5–1·0 mg/day; (2) subcutaneous implantation under ether anaesthesia of a single 20 mg fused pellet of oestradiol (Organon). In some animals with oestradiol implants, concurrent implantation of a single 100 mg fused progesterone pellet (Organon) was also performed.

Results

Sensitivity to acetylcholine in non-pregnant animals

As reported previously (Bell, 1968) the sensitivity to acetylcholine (ACh) of arteries taken from virgin animals, whether in dioestrus or oestrus, was extremely low (Table 1, Figure 2). Only weak responses to 50 ng ACh were obtained in 2 out of 10 arteries tested during dioestrus and 4 out of 12 obtained during oestrus. The majority of vessels exhibited very small or no responses to doses of ACh as high as 10 μg.

Sensitivity to acetylcholine during pregnancy

The time course of the development of sensitivity to ACh during gestation was investigated using perfused arteries from animals at various stages of pregnancy

TABLE 1. *Responsiveness to acetylcholine of uterine arteries from virgin guinea-pigs during dioestrus and oestrus and from primiparous pregnant guinea-pigs.*

	Sensitivity to Acetylcholine*		No. preparations
	Range	Mean±S.E.	
Dioestrus	0–15	2·0±1·5	10
Oestrus	0–13	2·3±1·2	12
Pregnancy (56 days)	13–66	31·5±7·0	6

*Ratio between response to acetylcholine (50ng) and response to noradrenaline (0·5μg), expressed as a percentage.

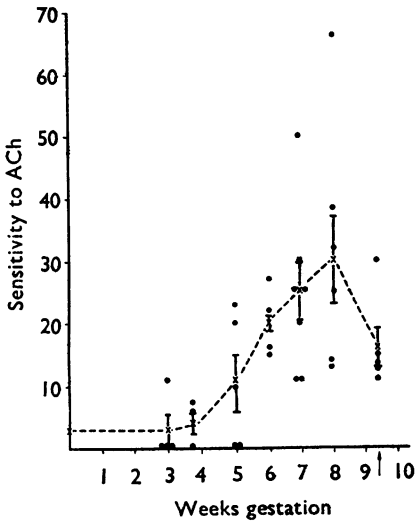

FIG. 2. Relation between the stage of gestation and the sensitivity to acetylcholine of the perfused uterine arteries of primiparous guinea-pigs. The sensitivity is derived from the ratio between amplitudes of responses to acetylcholine (50 ng) and noradrenaline (0·5 μg), expressed as a percentage. Each black dot represents a single preparation, and the vertical black bars are standard errors of the means (×). The arrow indicates the day of parturition (day 65).

(Figure 2). The sensitivity did not increase appreciably above non-pregnant values until 25 days of gestation or slightly later. After this time it rose progressively to reach a maximum at around day 56 of gestation. At this time the sensitivity of the uterine artery to ACh was about 10 times higher than that of a non-pregnant guinea-pig. Subsequently the sensitivity declined to about 5 times the non-pregnant value at term. In animals bearing foetuses in only one uterine horn there was no consistent difference in sensitivity between the arteries supplying the pregnant and the non-pregnant horns.

Sensitivity to acetylcholine after oestrogen treatment

The treatment of virgin guinea-pigs with intramuscular injections of oestradiol (0·5 mg/day) for 14 days caused an increase in the responsiveness of the uterine artery to ACh. The sensitivity developed was similar to that seen in the second half of pregnancy (Table 2). After 7 days' treatment, the degree of sensitization was less, while 3 days' treatment had no effect (Table 2). After 3 days' treatment with a larger dose of oestradiol (1 mg/day) good responsiveness was developed in 6 out

TABLE 2. *Comparison of responsiveness to acetylcholine of uterine arteries from virgin guinea-pigs in natural oestrus with that of arteries from animals treated with oestrogens.*

Treatment Hormone	Duration	Sensitivity to Acetylcholine* Range	mean ± S.E.	No. Preparations
Natural oestrus		0–13	2·3 ± 1·2	12
Oestradiol-17β Benzoate 0·5 mg/day	3 days	0	0	4
	7 days	14,25	19	2
	14 days	13–50	28 ± 4·5	6
Oestradiol-17β Valerate 0·5 mg/day	3 days	0	0	7
	7 days	10–22	16 ± 1·4	5
Oestradiol-17β Valerate 1 mg/day	3 days	0–50	20 ± 5·9	9
Oestradiol-17β Implants 0·047 mg/day†	7 days	0– 3	3 ± 1·4	4
	2 weeks	0–55	30 ± 10·0	5
	3–6 weeks	3–50	21 ± 3·8	13
Oestradiol-17β Implants 0·047 mg/day† + Progesterone Implants 0·68 mg/day†	3–4 weeks	0–45	16 ± 4·0	10

*Ratio between response to acetylcholine (50ng) and response to noradrenaline (0·5μg) expressed as a percentage. The animals were killed on the day after the last injection; †Mean absorption rates.

of 9 arteries tested. Three arteries did not respond to 50 ng ACh, but differed from non-treated controls in that they exhibited appreciable dilator responses to slightly higher doses of ACh (0·1–0·4 μg).

On the basis of dry weight alterations, it was calculated that subcutaneous implants of oestradiol were absorbed at rates between 16 and 80 μg/day (mean ± S.E., 47 ± 4 μg/day). One week of implantation caused no development of sensitivity to ACh, but treatment for two weeks or longer resulted in a degree of sensitization to ACh similar to that which occurred during pregnancy (Table 2).

Oestrogen treatment was associated with rupture of the vaginal closure membrane, flushing of the vaginal mucosa and the uterus, massive uterine hypertrophy and involution of the usually prominent intraabdominal fat pads associated with the broad ligaments. These last two effects became progressively more pronounced as exposure to the oestrogen was prolonged up to 6 weeks. However the degree of sensitization to ACh appeared to be maximal after 2 weeks. There was no correlation between the degree of sensitivity to ACh developed in individual animals and the rate of oestrogen absorption. In one animal which had an oestrogen implant for 4 weeks, neither the arterial responsiveness to ACh nor gross pelvic morphology was affected, although the apparent rate of oestrogen absorption derived from weight reduction of the pellet indicated an absorption rate of 33 μg/day.

Interaction of oestrogen and progesterone

The implants of progesterone were absorbed at rates between 0·43 and 0·86 mg/day (mean ± S.E., 0·68 ± 0·05 mg/day). In the presence of progesterone the mean degree of oestrogen-induced responsiveness to ACh was slightly reduced (from 21% to 16%) but remained appreciable, and in some animals it was as high as normally seen with oestrogen administration alone (Table 2). There was no

correlation between the degree of sensitization to ACh achieved and the relative absorption rates of oestrogen and progesterone in different animals. In contrast to this weak antagonism by progesterone of the arterial effect of oestrogen, the usual oestrogen effects on vagina and uterus and on the intraabdominal fat deposits were entirely or almost entirely prevented by the presence of progesterone.

Discussion

The previously reported phenomenon of sensitization of the guinea-pig parametrial artery to ACh during pregnancy (Bell, 1968) has been further investigated by using a method which provides quantitative data for the responsiveness of the arteries to ACh at various stages of pregnancy and after hormone treatment. The experiments have shown that appreciable sensitivity of the uterine artery to ACh begins to develop slightly before mid-term, reaches a maximum at about 56 days of pregnancy and then declines somewhat prior to parturition.

The factor responsible for this development of responsiveness to ACh appears to be the increase in circulating oestrogens as chronic administration of oestradiol by intramuscular injection or by subcutaneous implant caused the development of a similar degree of responsiveness. A causal relation between the two parameters is further indicated by the striking parallelism in time of the development of the sensitivity to ACh and the increase of plasma concentration of oestrogens during pregnancy as estimated by Challis, Heap & Illingworth (1971) (Figure 3).

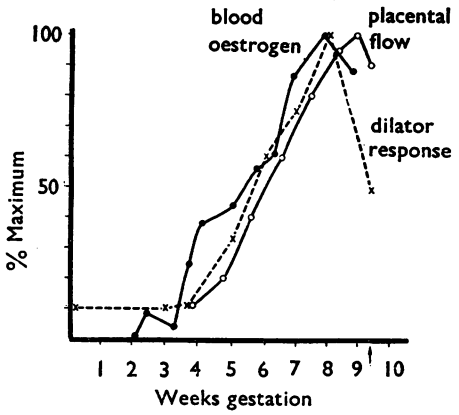

FIG. 3. Comparison of the chronological changes during gestation in the guinea-pig of the total unconjugated plasma oestrogens (●——●) (derived from the figures of Challis *et al.* (1971), placental blood flow (○——○) (replotted from Flexner & Gellhorn (1942)) and sensitivity to acetylcholine of the uterine arties (×——×). The sensitivity is derived from the ratio between amplitudes of responses to acetylcholine (50 ng) and noradrenaline (0·5 μg). The arrow indicates the day of parturition (day 65).

The rate of development of responsiveness to ACh during oestrogen administration was dose-dependent. Appreciable sensitivity appeared after 3 days of treatment with 1 mg/day oestradiol, but only after 7 days of treatment with 0·5 mg/day. Oestradiol implants which were absorbed at a rate of about 0·05 mg/day had to be present for 2 weeks. The doses of oestradiol necessary to produce arterial responsiveness to ACh were far in excess of the 2·6 μg/day calculated to be the

maximal daily production rate of oestradiol-17β during pregnancy in the guinea-pig (Challis *et al.*, 1971). However oestradiol-17β has been shown to constitute less than 10% of the total unconjugated plasma oestrogens in the pregnant guinea-pig (Challis, *et al.*, 1971). It may be that the low potency of exogenous oestradiol-17β on arterial ACh sensitivity reflects the fact that some other oestrogenic substance is the physiological mediator of the response.

The original observation that the guinea-pig parametrial artery responded to ACh and to cholinergic dilator nerve stimulation only during the latter half of pregnancy led to the suggestion that these nerves may be specifically concerned with the massive increase in local blood demand over this period of foetal growth (Bell, 1968).

Subsequently arteriography in anaesthetized guinea-pigs has shown the existence during late pregnancy of a tonic dilator influence on the parametrial vasculature which is abolished by atropine (Bell & Brown, 1971). In this context it is of interest that the time course of development of the sensitivity of the uterine artery to ACh during pregnancy is similar to the time course of enhancement of placental blood flow as calculated by Flexner & Gellhorn (1942), as well as to that of oestrogen production (Figure 3).

In many of their actions on the body, progestins and oestrogens are antagonistic, as evidenced in the present study by the prevention in the presence of progesterone of the uterine hypertrophy, vaginal and uterine flushing and catabolism of adipose tissue normally associated with chronic oestrogen treatment. In contrast, the oestrogenic stimulation of arterial sensitivity to ACh was largely unaffected by progesterone. In view of the probable functional significance of the cholinergic dilator system during pregnancy this is hardly surprising, as sensitization occurs at a stage of gestation when the plasma progesterone level is already at its maximum (Challis *et al.*, 1971).

Dependence of dilator responsiveness of the uterine artery to ACh on circulating oestrogens is not restricted to the guinea-pig. Graham & Sani (1971) have recently demonstrated that while the perfused uterine arteries of untreated rabbits constrict in response to ACh, those from rabbits treated for 10 days with high doses of stilboestrol respond to ACh with dilatation. In contrast, the uterine arteries of the sheep and of the dog appear to be able to dilate in response to ACh in the absence of oestrogenic stimulaion (Greiss, Gobble, Anderson & McGuirt, 1967; Ryan, Clarke & Brody, 1972).

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