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Effects of progesterone therapy on the stilboestrol-induced sensitivity of isolated rat uterus preparations

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1. Progesterone treatment (50 mg/kg) in ovariectomized rats antagonizes the effects of stilboestrol 100 μg/kg on the vaginal mucosa and uterine weight; but the sensitivity to acetylcholine, oxytocin and 5-hydroxytryptamine is increased by factors 4, 11 and 3, respectively.

2. In intact animals a similar progesterone treatment (50 mg/kg) antagonizes the effects of stilboestrol 100 μg/kg on vaginal smears, uterine weight and uterine sensitivity. The uterine sensitivity to acetylcholine, oxytocin and 5-hydroxytryptamine is, however, decreased by a factor of 2, 1.4 and 17.7, respectively.

3. These experiments show that the ovary is essential for the desensitizing effects of progesterone. The suggestion is put forward that another substance is released from the ovary by progesterone.

Khan & Qureshi (1967a, b) observed that stilboestrol could not produce its sensitizing effect on the rat uterus after reserpine therapy. Ansary (1965) and Khan & Shariff (1967) failed to observe the desensitizing effect of reserpine on ovariectomized and on hypophysectomized female rats. Fajer & Barraclough (1966) reported a significant increase in the concentration of progesterone in ovarian venous blood in rats during pseudopregnancy induced by reserpine. On the basis of these observations Khan & Qureshi (1967b) postulated that the reduction in uterine sensitivity after reserpine therapy was due to a high level of progesterone secreted by the persistent corpora lutea.

The action of progesterone on the myometrium is not yet clearly understood for most species. *In vitro* progesterone has been shown to inhibit human (Kumar, Goodno & Barnes, 1962) and rat myometrium (Saldivar & Malton, 1966). *In vivo* progesterone produces effects on the uteri varying from inhibition in rabbits (Csapo, 1956) to excitation in the sow (Knifton, 1962, cited by Saldivar & Malton, 1966).

Thiersch, Landa & West (1959), Goto & Csapo (1959) and Kuriyama & Csapo (1961) observed a higher membrane potential of the myometrial cell, blockage in the propagation of the electrical activity and asynchrony between electrical and

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mechanical activity in rat and rabbit myometrium at the advanced stage of pregnancy. But Kao & Nishiyama (1964) and Saldivar & Malton (1966) showed that parenteral administration of progesterone to ovariectomized rats and rabbits had no effect on the electrical and the mechanical activity of the myometrium.

Similarly, results of studies on the human myometrium are also controversial (Caldeyro-Barcia & Posiero, 1959 ; Bengtssen & Csapo, 1962).

The observation of Khan & Qureshi (1967a, b) led to the belief, without experimental evidence, in the important role of progesterone in monitoring rat uterine sensitivity and there was thus a need to study the effect of progesterone therapy on the oestrogen-induced sensitivity of rat uterus.

Methods

Sixty female virgin Sprague-Dawley strain rats (180–230 g) with a normal and regular (4 day) oestrus cycle were used. Vaginal smears were obtained with a pipette and examined daily throughout the experiment.

Ovariectomy

Rats were spayed under ether anaesthesia on the first day of dioestrus. Progesterone was administered intraperitoneally in doses varying from 4 to 100 mg/kg body weight on the first day of dioestrus, 5–6 hr after ovariectomy, and repeated on the third day of dioestrus. Stilboestrol (100 μg/kg) was administered intraperitoneally to all the animals on the third day of dioestrus, 3 hr after the second dose of progesterone. The animals were killed 18 hr later. The unoperated rats were similarly treated with progesterone (50 mg/kg) and stilboestrol. All the uterine horns along with the body of the uterus were weighed.

Isolated rat uterus preparations

A 2 cm length of uterine horn was suspended in a 5 ml. organ bath containing de Jalon's solution at 30° C. ; a similar bath and solution were used by Khan & Qureshi (1967b). Oxytocic drugs were added to the bath and the contractions of the horn were recorded isotonically on a kymograph with a frontal point writing lever, at a magnification of 4. The dose-response curve of acetylcholine (ACh), oxytocin and 5-hydroxytryptamine (5-HT) were recorded. If the dose-response curve of oxytocin and 5-HT was found parallel to that of acetylcholine, then the relative potency of oxytocin and 5-HT was estimated by a 3-point assay method and the results were expressed as equipotent molar ratios in terms of ACh as described by Barlow & Khan (1959).

Drugs

Doses of acetylcholine chloride, 5-hydroxytryptamine creatinine sulphate were expressed as bases and of oxytocin (syntocinon) as 2 μg = 1 unit. Diethylstilboestrol was originally dissolved in sesame oil but further diluted in arachis oil. The lutocycline brand of progesterone (Ciba Laboratory, Pakistan, Ltd.) was used.

Results

Stimulant activity of oxytocic drugs on isolated rat uterus preparations

Group I (Normal rats receiving progesterone 50 mg/kg):

All the uterine horns from twelve rats showed intense spontaneous activity; this activity did not subside in four uteri even after 4 hr and they were discarded.

(i) *Acetylcholine.* Responses were obtained in the dose range of 0.4–8 μg/5 ml. (Table 1); the dose-response curve was linear.

(ii) *Oxytocin.* Stimulation occurred with a dose range of 2–32 ng/5 ml. (Table 1). The dose-response curve was parallel to that of acetylcholine. The equipotent molar ratio of oxytocin to acetylcholine was 0.12 ± 0.01 (S.E.) (Table 1).

(iii) *5-Hydroxytryptamine.* The uteri were contracted by doses ranging from 0.2 to 6.4 μg/5 ml. (Table 1). Tachyphylaxis occurred when higher doses were applied. The dose-response curve was parallel to that of acetylcholine. The equipotent molar ratio of 5-hydroxytryptamine to acetylcholine was 190.6 ± 22.4 (S.E.) (Table 1).

Group II (Ovariectomized rats receiving progesterone):

Sub-group A; progesterone 4 mg/kg. All the horns from the twelve uteri showed variable spontaneous activity which subsided within 0.5 hr of suspension.

(i) *Acetylcholine.* The uteri responded in the dose range of 0.04–6.4 μg/ml. (Table 1). The dose-response curve was linear.

(ii) *Oxytocin.* The uteri were stimulated with 0.5–16 ng/ml. (Table 1). The equipotent molar ratio of oxytocin to acetylcholine was 0.13 ± 0.02 (S.E.) (Table 1).

(iii) *5-Hydroxytryptamine.* The uteri were stimulated by doses ranging from 0.02 to 1.6 μg/5 ml. (Table 1). Still higher doses produced tachyphylaxis. The dose-response curve was parallel to that of acetylcholine. The equipotent molar ratio of 5-hydroxytryptamine to acetylcholine was 66.8 ± 2.13 (S.E.).

Sub-group B; progesterone 50 mg/kg. All the horns from twelve uteri showed a marked spontaneous activity, when suspended in the bath, which continued for 90 min. Horns from the two uteri showed intense activity up to 3 hr and were discarded.

(i) *Acetylcholine.* The uteri responded to acetylcholine in the dose range of 0.03–16 μg/5 ml. (Table 1).

(ii) *Oxytocin.* The uteri were stimulated by 0.1–16 ng/5 ml. (Table 1). The equipotent molar ratio of oxytocin to acetylcholine was 0.13 ± 0.02 (S.E.) (Table 1).

(iii) *5-Hydroxytryptamine.* The tissue responded to doses ranging from 0.01 to 12.8 μg/5 ml. (Table 1). Higher doses produced tachyphylaxis. The equipotent molar ratio of 5-hydroxytryptamine to acetylcholine was 49.1 ± 2.1 (S.E.) (Table 1).

Sub-group C; progesterone 100 mg/kg. Animals receiving progesterone in doses of 100 mg/kg were more or less anaesthetized. Horns from twelve uteri showed marked spontaneous activity, but the activity continued for 3 hr in four horns, which were discarded.

(i) *Acetylcholine.* The uteri were stimulated by doses ranging from 0.1–4 μg/5 ml. (Table 1).

TABLE 1. *Sensitivity of rat isolated uteri to oxytocin (Ox) and 5-hydroxytryptamine (5-HT), as compared with acetylcholine (ACh), and changes in uterine weight, after various tretments*

Groups	Rat treatments	Range of effective (minimal and maximal) doses of stimulant drugs in 5 ml. bath			Average equipotent molar ratios ±S.E.		Average weight of the uteri mg±S.E.
		ACh	Ox	5-HT	Ox/ACh	5-HT/ACh	
I	Unoperated, progesterone 50 mg /kg, and stilboestrol-treated	0·4–8 μg	2–32 ng	0·2–6·4 μg	0·12±0·01 (6)	190·6±22·4 (6)	395±22·7 (6)
II A	Ovariectomized + Progesterone 4 mg/kg + Stilboestrol	0·04–6·4 μg	0·5–16 ng	0·02–1·6 μg	0·13±0·02 (6)	66·8±0·13 (6)	454±20·1 (6)
II B	Ovariectomized + Progesterone 50 mg/kg + Stilboestrol	0·03–16 μg	0·1–16 ng	0·01–12·8 μg	0·13±0·03 (6)	49·1±2·1 (6)	440±11·8 (6)
II C	Ovariectomized + Progesterone 100 mg/kg + Stilboestrol	0·1–4 μg	0·2–32 ng	0·01–1·28 μg	0·14±0·01 (6)	48·1±6·7 (6)	386±20 (7)
III	Normal saline	0·2–40 μg	0·2–80 ng	0·05–4 μg	0·17±0·02 (7)	21·6±1·9 (6)	471±17·1 (7)

Acetylcholine was kept as 100 in each group. Numbers in brackets indicate numbers of observations. The sensitivity of uteri to acetylcholine varies in the various different groups, so the comparison is only valid for each group.

(ii) *Oxytocin.* The minimum to maximum dose range to stimulate the uterus was 0.2–32 ng/5 ml. (Table 1). The equipotent molar ratio of oxytocin to acetylcholine was 0.14 ± 0.01 (S.E.) (Table 1).

(iii) *5-Hydroxytryptamine.* The tissue responded in the dose range 0.01–1.28 μg/5 ml. (Table 1). The equipotent molar ratio of 5-hydroxytryptamine to acetylcholine was 48.1 ± 6.7 (S.E.) (Table 1).

Group III (Ovariectomized rats in oestrus induced by stilboestrol 100 μg/kg):

(i) *Acetylcholine.* The dose range of acetylcholine producing contractions was 0.2–32 μg/5 ml. (Table 1).

(ii) *Oxytocin.* Contractions were elicited by 0.2–80 ng/5 ml. (Table 1). The equipotent molar ratio of oxytocin to acetylcholine was 0.17 ± 0.22 (S.E.) (Table 1).

(iii) *5-Hydroxytryptamine.* The uteri responded to 0.05–4 μg/5 ml. (Table 1). Tachyphylaxis was observed with higher doses. The equipotent molar ratio of 5-hydroxytryptamine to acetylcholine was 21.6 ± 1.9 (S.E.) (Table 1).

Examination of vaginal smears

Group I

The vaginal smears showed a preponderance of leucocytes and nucleated epithelial cells, indicating that stilboestrol had failed to induce vaginal oestrus.

Group II

Sub-group (A). Before the administration of stilboestrol the vaginal smears showed an abundance of leucocytes, but 18 hr after stilboestrol therapy the presence of cornified cells indicated oestrus. In both sub-groups (*A*) and (*C*) the vaginal smears showed a preponderance of leucocytes and nucleated epithelial cells as in group I.

Group III

Following ovariectomy and before the administration of stilboestrol the vaginal smears showed an abundance of leucocytes; 18 hr after stilboestrol therapy cornified cells were observed, indicating oestrus.

Uterine weight

The uterine weights in mg are given in Table 1. The value for group I (395 ± 22.7) differs significantly ($P<0.05$) from that (471 ± 17.1) of group III (control group). This indicates that progesterone therapy antagonizes the uterine weight gain induced by stilboestrol therapy only when the ovary is present.

Discussion

The uterine sensitivity of rats in group III (ovariectomized and stilboestrol-treated in this work agrees with a similar group in Ansary's (1965) work. Rats receiving progesterone 4 mg/kg in group II showed an insignificant reduction in uterine weights. The vaginal smears showed cornification. The uterine sensitivity to acetylcholine, oxytocin and 5-hydroxytryptamine was increased 5, 6, and 1.7

times, respectively, as compared with control group III (Table 2), indicating that progesterone therapy has facilitated the response to oxytocic drugs. Progesterone in doses of 50 mg/kg (group II, *B*) produced an insignificant reduction in the uterine weights ($P>0.05$), but the effect of stilboestrol on the vaginal mucosa was antagonized. The uterine sensitivity to acetylcholine, oxytocin and 5-hydroxytryptamine was increased (Table 2) 7, 9.9 and 3 times, respectively, indicating facilitation of the effects of stilboestrol. Although progesterone 100 mg/kg body weight (group II, *C*) induced toxic effects resembling anaesthesia, it still produced facilitation of the uterine sensitivity to oxytocic drugs. The maximum facilitation on uterine sensitivity to the three oxytoxic drugs was seen with 50 mg/kg doses of progesterone. Rats with intact ovaries and treated with 50 mg/kg doses of progesterone (group I), however, also showed absence of vaginal oestrus. The uterine weights in group I showed a significant reduction ($P<0.05$) as compared with group III. The uterine sensitivity to acetylcholine, oxytocin and 5-hydroxytryptamine was decreased 2, 1.4 and 17 times (Table 2), respectively. This shows that progesterone therapy in intact rats produced a marked antagonism of stilboestrol-induced gain in uterine weight and increase in uterine sensitivity.

Progesterone has produced pseudopregnancy in rats after a single dose of 50 mg/kg body weight (Everett, 1963) and a similar dose given in divided doses on the first and second days of dioestrus (Khan & Bernstorf, 1964). In this work two doses of progesterone, 50 mg/kg on the first and third day of dioestrus, in the rats with intact ovaries produced a marked reduction in stilboestrol-induced sensitivity and uterine weight gain, indicating the active role of the ovaries in reducing uterine sensitivity during progesterone-induced pseudopregnancy. Progesterone may produce the desensitizing effect in the uterine sensitivity by liberating another chemical substance, probably from the corpora lutea, which may be responsible for its desensitizing effect on the uterus. This effect of progesterone, like that of reserpine, may be of central origin.

The suggestion of Khan & Qureshi (1967b) that reserpine produces desensitization of the uterus by secreting more progesterone available from the corpus luteum needs reconsideration because of these results.

TABLE 2. *Rat isolated uteri: relative equipotent molar ratios of oxytocin (Ox) and 5-hydroxytryptamine (5-HT) to acetylcholine (ACh)*

Group			Rat treatments		Drug ratios		
					ACh	Ox	5-HT
I			Unoperated, progesterone 50 mg/kg, and stilboestrol-treated.	Stilboestrol 100 μg/kg	200	0·24	380
II	*A*	Ovariectomized + Progesterone	4 mg/kg	Stilboestrol 100 μg/kg	0·20	0·026	13·3
	B	Ovariectomized + Progesterone	50 mg/kg	Stilboestrol 100 μg/kg	14·3	0·018	7·01
	C	Ovariectomized + Progesterone	100 mg/kg	Stilboestrol 100 μg/kg	50	0·07	24
III		Ovariectomized	Normal saline	Stilboestrol 100 μg/kg	100	0·17	21·6

Value of 100 has been arbitrarily fixed for acetylcholine in group III. Comparison is valid for all groups.

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