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Study of the effects of progesterone therapy on the stilboestrol-induced sensitivity of isolated uteri of hypophysectomized rats

I. KHAN*, Z. QURESHI AND M. HAQ

Department of Pharmacology and Therapeutics, Jinnah Postgraduate Medical Centre, Karachi

Summary

1. In intact rats progesterone (50 mg/kg) antagonized the effect of stilboestrol (100 μg/kg) on the vaginal smears and the uterine sensitivity. The uterine sensitivity to acetylcholine, oxytocin and 5-hydroxytryptamine was decreased by 6·8, 64 and 14·8 times, respectively, as compared with uteri removed after stilboestrol injections.

2. Progesterone therapy in hypophysectomized rats also antagonized the effect of stilboestrol on the vaginal mucosa and on the uterine sensitivity to drugs. The sensitivity to acetylcholine, oxytocin and 5-hydroxytryptamine was decreased by 6·2, 50 and 14·5 times, respectively. Similar results were obtained on uteri removed from sham-hypophysectomized rats.

3. On the basis of these results it is suggested that the hypophysis does not play any part in the desensitization of the myometrium to the oxytocic drugs and in the changes found in the vaginal mucosa after progesterone therapy.

Introduction

Khan & Qureshi (1967a, b) observed that reserpine prevented the marked increase produced by stilboestrol in the sensitivity of isolated rat uteri preparations to oxytocic drugs. They considered it to be due to excessive release of progesterone from the persistent corpora lutea. This effect of reserpine disappeared after ovariectomy (Khan, Qureshi & Ansary, 1969) and hypophysectomy (Khan & Shariff, 1967). Fajar & Barraclough (1967) reported a significant increase in the concentration of progesterone in ovarian venous blood in rats during pseudopregnancy induced by reserpine. Progesterone therapy in ovariectomized rats increased the sensitivity of the rat uteri preparations to oxytocic drugs, but in animals with intact ovaries it produced a marked reduction in uterine sensitivity, indicating an active role for the ovaries in reducing uterine sensitivity in such circumstances (Khan & Ahmed, 1968, 1969). This effect of progesterone, like that of reserpine, may be of central origin. In the present work the effect of progesterone therapy on the uteri of hypophysectomized rats was studied to investigate the role of the hypophysis. A preliminary account of the work was presented at the IV International Congress on Pharmacology.

* Present address: Drugs Research Institute, National Health Laboratories, Islamabad.

Methods

Ninety-two female virgin Sprague-Dawley rats (200–250 g) showing a normal and regular oestrus cycle for 4–5 days were used. The vaginal smears were obtained with a pipette and examined daily throughout the experiment.

Hypophysectomy and treatment of animals

Rats were hypophysectomized and sham-hypophysectomized through a parapharyngeal approach (D'Amour & Blood, 1954) under ether anaesthesia on the first day of dioestrus. Two doses of progesterone each of 50 mg/kg body weight were administered intraperitoneally (on the first day of dioestrus) 6 hr after operation, and 48 hr later (on the third day of dioestrus). Stilboestrol (100 mg/kg) was administered intraperitoneally on the third day of dioestrus, 3 hr after the second dose of progesterone, and animals were killed 18 hr later. The unoperated rats also received progesterone and stilboestrol.

The rats of the first group received only stilboestrol on the first day of dioestrus and were killed 18 hr later.

Isolated rat uterus preparations

A 2·5 cm length of the uterine horn was suspended in a 5 ml organ bath containing De-Jalon's solution at 30° C. The oxytocic drugs were added to the bath and the contractions were recorded isotonically on a kymograph with a frontal writing point lever with a magnification of 4. The dose response curves of acetylcholine, oxytocin and 5-hydroxytryptamine were recorded as described by Khan & Ahmed (1969). When the dose response curves for oxytocin and 5-hydroxytryptamine were found to be parallel to that of acetylcholine, the relative potency of oxytocin and 5-hydroxytryptamine were estimated by an 8-point assay method, and the results were expressed as equipotent molar ratios in terms of acetylcholine as described by Barlow & Khan (1959).

Drugs

Doses of acetylcholine chloride and 5-hydroxytryptamine creatinine sulphate are expressed as bases. 2 μg of Syntocinon (oxytocin) represent one unit. Diethylstilboestrol was diluted with arachis oil and the lutocyclin brand of progesterone was used.

Results

Stimulant activity of oxytocic drugs

Group I : normal rats receiving stilboestrol

The uteri were thick, hyperaemic and showed prominent blood vessels on their surface. The horns were distended with fluid ; they showed peristalsis-like movement *in situ* as well as in a Petri dish, and assumed a coil-like shape. On suspension in the organ bath, the tissues did not show any spontaneous activity.

Acetylcholine. The responses were obtained in doses ranging from 48 to 620 ng/5 ml (Table 1). The dose response curve was linear.

Oxytocin. The tissues were stimulated in a dose range of 100–533 pg/5 ml (Table 1). The dose response curve was parallel to that of acetylcholine. The equipotent molar ratio of oxytocin to acetylcholine was $0{\cdot}15 \pm 0{\cdot}014$ (S.E.).

5-Hydroxytryptamine. The tissues responded within a dose range of 20–320 ng. The equipotent molar ratio of 5-hydroxytryptamine to acetylcholine was $60 \pm 10{\cdot}1$ (S.E.).

Group 2 : normal rats receiving progesterone and stilboestrol

All the tissues showed intense spontaneous movement on suspension in the bath. The spontaneous activity subsided within 0·5 hr.

Acetylcholine. The tissues responded to 0·33–5·5 μg/5 ml (Table 1). The dose response curve was linear.

Oxytocin. The dose range which stimulated the tissues was 3–50 ng/5 ml (Table 1). The equipotent molar ratio of oxytocin to acetylcholine was $0{\cdot}14 \pm 0{\cdot}07$ (S.E.).

5-Hydroxytryptamine. The dose range which stimulated the tissue was 0·25–4 μg/5 ml (Table 1). The equipotent molar ratio of 5-hydroxytryptamine to acetylcholine was $130 \pm 40{\cdot}4$ (S.E.).

Group 3 : hypophysectomized rats receiving progesterone and stilboestrol

These tissues did not show any spontaneous activity.

Acetylcholine. The average dose range which stimulated the tissue was 0·3–3·2 μg/5 ml (Table 1). The dose response curve was linear.

TABLE 1. *Sensitivity of rat isolated uteri to 5-hydroxytryptamine (5-HT) and oxytocin (Ox) as compared with acetylcholine (ACh) after various treatments*

		Range of effective doses (minimal and maximal) of stimulant drugs in 5 ml bath			Average equipotent molar ratios ± (S.E.)	
Groups	Treatment	ACh	Ox	5-HT	Ox/ACh	5-HT/ACh
1.	Stilboestrol 100 μg/kg	48–620 ng	100–533 pg	20–320 ng	$0{\cdot}15 \pm 0{\cdot}014$ (6)	$60 \pm 10{\cdot}1$ (6)
2.	Progesterone 50 mg/kg + stilboestrol 100 μg/kg	0·33–5·5 μg	3–50 ng	0·25–4 μg	$0{\cdot}14 \pm 0{\cdot}70$ (6)	$130 \pm 40{\cdot}4$ (6)
3. Hypophysectomized	Progesterone 50 mg/kg + stilboestrol 100 μg/kg	0·30–3·2 μg	2–40 ng	0·2–3·2 μg	$0{\cdot}12 \pm 0{\cdot}43$ (8)	$140 \pm 20{\cdot}6$ (6)
4. Sham-hypophysectomized	Progesterone 50 mg/kg + stilboestrol 100 μg/kg	0·31–5·8 μg	3–53 μg	0.25–4·9 μg	$0{\cdot}13 \pm 0{\cdot}08$ (6)	127 ± 60 (6)

Acetylcholine was taken as 100 in each group.
Numbers in parenthesis indicate number of observations.
As the sensitivity of the uteri for acetylcholine varies from group to group, a comparison is only valid within each group.
S.E., standard error.

Oxytocin. The tissues were stimulated by the dose range 2–40 ng/5 ml (Table 1). The equipotent molar ratio of oxytocin to acetylcholine was $0{\cdot}12 \pm 0{\cdot}04$ (S.E.).

5-Hydroxytryptamine. The tissues responded to a dose range 0·2–3·2 μg/5 ml (Table 1). The equipotent molar ratio of 5-hydroxytryptamine to acetylcholine was $140 \pm 20{\cdot}6$ (S.E.).

Group 4 : sham-hypophysectomized rats receiving progesterone and stilboestrol

Acetylcholine. The average effective dose range varied from 0·31–5·8 μg/5 ml (Table 1). The dose response curve was linear.

Oxytocin. The tissues were stimulated within an average dose range of 3–53 ng/5 ml (Table 1). The equipotent molar ratio of oxytocin to acetylcholine was $0{\cdot}13 \pm 0{\cdot}08$ (S.E.).

5-Hydroxytryptamine. The average dose range which produced contraction of the tissues varied from 0·25–4·8 μg/5 ml (Table 1). The equipotent molar ratio of 5-hydroxytryptamine to acetylcholine was 127 ± 60 (S.E.).

Examination of the vaginal smears

The vaginal smears of all the groups of rats (normal hypophysectomized and sham-hypophysectomized) treated with progesterone and stilboestrol showed abundant leucocytes, indicating the absence of stilboestrol effect. The groups of normal rats receiving stilboestrol alone showed cornified cells.

Discussion

Khan & Ahmed (1969) have demonstrated that progesterone therapy in the dose range producing pseudopregnancy in rats with intact ovaries produces desensitization of the isolated uteri after stilboestrol. They have clearly shown that the ovary is essential for the desensitizing effect of progesterone on uteri. The mechanism suggested was through liberation from the corpora lutea of a chemical substance other than progesterone, which desensitizes the uteri. This effect of progesterone therapy was considered to be of central origin, similar to reserpine. Groups of intact

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

Groups	Treatment	Drug ratios		
		ACh*	5-HT	Ox
1.	Stilboestrol 100 μg/kg	*100	60	0·015
2.	Progesterone 50 mg/kg + stilboestrol 100 μg/kg	687·5	893·7	0·96
3. Hypophysectomized	Progesterone 50 mg/kg + stilboestrol 100 μg/kg	625	875	0·75
4. Sham-hypophysectomized	Progesterone 50 mg/kg + stilboestrol 100 μg/kg	645	793·7	0·83

* The value of 100 has been arbitrarily fixed for acetylcholine in group 1. Comparison is valid for all groups.

rats receiving progesterone and stilboestrol, in the study of Khan & Ahmed (1969), showed a reduction in the sensitivity of uteri to acetylcholine, oxytocin and 5-hydroxytryptamine by factors of 2, 1·4 and 17·7, respectively, as compared with tissues removed from intact rats receiving stilboestrol alone.

In this work uteri removed from hypophysectomized rats receiving both progesterone and stilboestrol also showed reduction in their sensitivity to acetylcholine, oxytocin and 5-hydroxytryptamine by factors of 6·2, 50 and 14·5, respectively, when compared with similar tissues of intact rats, and thus showed a uniform degree of desensitization. The vaginal smear in both the hypophysectomized and sham-hypophysectomized rats also showed absence of the stilboestrol effect (cornification) as a result of progesterone therapy. This indicates that progesterone produced desensitization of rat uteri and prevented cornification in the vaginal mucosa after stilboestrol therapy in the absence of pituitary glands, thus showing that the effect of progesterone on the uteri is not dependent on the pituitary but on the ovary.

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