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Effects of reserpine on the sensitivity of rat isolated uteri preparations to oxytocic drugs

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Stimulant activity of the isolated uteri of female virgin rats to acetylcholine, 5-hydroxytryptamine, and oxytocin was examined quantitatively (4-point assay method) in anoestrus, induced oestrus (diethylstilboestrol 10 μg/100 g body weight injected subcutaneously), reserpinized and stilboestrol-treated reserpinized animals. Each group had 30 animals. Reserpine was administered intraperitoneally in two doses each of 1 mg/kg body weight on the first and third days of dioestrus. The sensitivity to acetylcholine, 5-hydroxytryptamine and oxytocin of the uteri in induced oestrus was increased by 20, 86 and 730 times respectively. In the group of animals receiving reserpine alone the experiment could not be made due to persistant spontaneous activity (even up to 2 hr) of the uterine tissue. In the stilboestrol-treated reserpinized group, the sensitivity to all the oxytocic drugs was as low as in anoestrous tissue. Thus, stilboestrol injection failed to increase the sensitivity of the rat uteri to oxytocic drugs or to produce changes in the vaginal smear pattern. The possibility of progesterone being present in the tissue due to persistance of corpora lutea after reserpine is put forward.

IN 1927, Knause (cited by Henry, Browne & Venning, 1950) observed an increase with oestrogen and a decrease with progesterone in the sensitivity of isolated uterus preparations of rabbits and guinea-pigs to posterior pituitary extract added to the organ bath. Stewart (1949) (cited by Krantz & Carr, 1961) also found an increase in sensitivity to oxytocin during oestrus and a decrease during dioestrus. Marshall (1962) observed a marked increase in the frequency and amplitude of action potentials set up by oxytocin in rat isolated uterus preparations removed from oestrogen-dominated animals. The rat-uterus preparation has been used for assay of oxytocic drugs (Pennefather, 1961).

Erspamer (1940) observed that rat isolated uteri in induced oestrus were sensitive to 5-hydroxytryptamine, (5-HT) and Erspamer (1953) found this to be 50 times more potent in oestrus than in anoestrus.

Reserpine delayed ovulation and caused amenorrhoea in monkeys (De Feo & Reynolds, 1956), and Barraclough & Sawyer (1959) observed pseudopregnancy following reserpine treatment in female rats.

Sensitivity of the atrium of rabbit to 5-HT was increased significantly after reserpinization (Trendelenburg, 1960).

Although many workers have referred to the changes produced in sensitivity of the uterine preparations to various oxytocic drugs by oestrogen, there is a need to investigate these changes quantitatively as one of the parameters of action of oestrogens. We have selected 5-HT, oxytocin and acetylcholine as stimulants for isolated uteri from virgin rats during anoestrus and after stilboestrol treatment.

Reserpine treatment of an animal modifies the responses of 5-HT and catecholamines on some tissues and we therefore examined the effect of reserpine on the responses of the uteri to 5-HT and the other two oxytocic drugs.

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Experimental

METHOD

Female virgin rats of 2–3 months of age, 200–250 g, from the Jinnah Postgraduate Medical Centre Colony were divided into 4 groups of 30 animals. The first group had animals both untreated and in anoestrus. In the second group, anoestrous animals were treated subcutaneously with stilboestrol (10 μ/100 g) 12 hr before death. Animals in the third group received reserpine intraperitoneally in two doses, each of 1 mg/kg, on the first and third day of dioestrus. The dose was estimated to be the maximum tolerated. Animals in the fourth group received stilboestrol 24 hr after the second dose of reserpine and 12 hr before death.

DRUGS

Doses of acetylcholine chloride and 5-hydroxytryptamine creatinine sulphate are expressed as bases. Oxytocin (Syntocinon) is expressed as 2 μg $\equiv$ 1 unit. Solution of diethylstilboestrol was prepared in sesame oil and further diluted in arachis oil.

PROCEDURE

Stimulant activity of the isolated pieces of uteri was estimated by suspending a 2 cm piece of uterine horn in a 5 ml organ bath, containing de Jalon solution at 30° (similar to that of Barlow & Khan, 1959). The contractions of the uterine tissues were recorded isotonically through a force transducer attached to a polygraph. The drug was left in contact with the tissue for 45 sec and a 2 min cycle was maintained throughout the experiment. Acetylcholine was used as a control.

Dose response curve. After allowing the tissue 30 min in the bath, a dose-response curve to acetylcholine was obtained. The entire dose-response curves (minimum to maximum) of each oxytocic drug were then recorded and compared with that of acetylcholine. If the dose-response curve of an oxytocic drug was found to be parallel to that of acetylcholine, then its potency was estimated by a 4-point assay method.

The 4-point assay was in accordance with the method used by Holton in 1948 (cited by Burn, Finney & Goodwin, 1950), for assay of oxytocin. The potencies were finally expressed as equipotent molar ratios in terms of acetylcholine (similar to Barlow & Khan, 1959).

Vaginal smear examination was by the Papanicolaou method and macroscopic examination of uteri in each group of the animals was also made.

Results

ANOESTROUS TISSUES (Group 1)

Four of 30 tissues used showed spontaneous activity, which subsided after 30 min in the bath. The tissue responded to doses of acetylcholine from 0·25 to 2 μg/5 ml. No tachyphylaxis or spontaneous activity, after washing out the drug, was observed with this drug. The dose-response

curve was linear. The potency of acetylcholine on this tissue was arbitrarily kept as 100 and the other drugs were compared with it.

The sensitivity to 5-HT was from 65 ng to 0·35 μg/5 ml. No spontaneous activity was observed after washing out the drug, but doses larger than these invariably produced tachyphylaxis. The entire dose-response curve of 5-HT was parallel to that of acetylcholine. The equipotent molar ratio of 5-HT as estimated and compared with acetylcholine was 42·0 $\pm$ 5·4 (Table 1); hence 5-HT was 2·4 times more potent than acetylcholine on a molar basis.

The effective dose range of oxytocin was from 0·8 to 8 ng/5 ml. It did not produce tachyphylaxis or spontaneous contractions after washing out the drug. The entire dose-response curve was parallel to that of acetylcholine. Its equipotent molar ratio was 0·11 $\pm$ 0·03 (Table 1). Thus oxytocin was 910 times more potent than acetylcholine.

TISSUES WITH INDUCED OESTRUS (Group 2)

All the 30 tissues used showed spontaneous activity, which subsided after 30 min in the bath. The tissue responded to acetylcholine in a dose range of 5 ng to 1 μg/5 ml. The dose-response curve of acetylcholine on this tissue was similar to the one in anoestrus except that this tissue was 20 times more sensitive to acetylcholine.

The tissue responded to 5-HT in doses varying from 30 to 340 ng/5 ml. Tachyphylaxis developed with higher doses. The entire dose-response curve was parallel to that of acetylcholine. The equipotent molar ratio of 5-HT compared with acetylcholine was 9·4 $\pm$ 0·6; hence 5-HT was 10·6 times more potent than acetylcholine on this tissue.

Oxytocin stimulated this tissue in doses ranging from 1·8 to 16 pg/5 ml. No tachyphylaxis or spontaneous activity was observed. The entire dose-response curve was parallel to that of acetylcholine. Its equipotent molar ratio was 0·003 $\pm$ 0·0002 (Table 1). Oxytocin was found to be 33300 and 3130 times more potent than acetylcholine and 5-HT respectively.

TISSUES AFTER RESERPINIZATION (Group 3)

Tissues from all the 30 animals when suspended in the bath showed persistent spontaneous activity for more than 2 hr. Addition of acetylcholine to the bath did not help. The experiments were therefore discontinued.

TISSUES FROM STILBOESTROL-TREATED RESERPINIZED ANIMALS (Group 4)

Uterine tissue of all the 30 animals showed spontaneous activity to variable degree when suspended in the bath. These movements persisted for up to 30 min. A few small doses of acetylcholine helped in settling the tissue. The sensitivity range of acetylcholine on this tissue was 0·3 to 4 μg/5 ml which is similar to that of anoestrous tissues.

The dose range of 5-HT was from 50 ng to 0·4 μg/5 ml and tachyphylaxis was not observed. No spontaneous activity was noted after washing out the drug. The entire dose-response curve was parallel to that of acetylcholine. Its equipotent molar ratio compared to acetylcholine was

TABLE 1. RAT ISOLATED UTERUS PREPARATION. STIMULANT ACTIVITIES OF 5-HYDROXYTRYPTAMINE (5-HT) AND OXYTOCIN (OX) COMPARED WITH ACETYLCHOLINE (Ach) ON VARIOUS TISSUES

Types of tissues	Range (minimal and maximal) of effective doses of stimulant drugs in a 5 ml bath			Equipotent molar ratios, Ach = 100		Potency ratios Ach = 1	
	Ach	5-HT	OX	5-HT	OX	5-HT	OX
Group 1. Rats in anoestrus	25 ng to 2 μg	65 to 350 ng	0·8 to 8 ng	42·0 ± 5·4 s.e. (10)	0·11 ± 0·03 s.e. (10)	2·4	909
Group 2. Induced oestrus	5 ng to 1 μg	30 to 340 ng	1 to 16 pg	9·4 ± 0·6 s.e. (10)	0·03 ± 0·0002 s.e. (10)	10·6	33300
Group 4. Stilboestrol and reserpine treated	300 ng to 4 μg	50 to 400 ng	0·8 to 6 ng	33·0 ± 3·0 s.e. (10)	0·06 ± 0·002 s.e. (10)	3	1670

Figures in parentheses indicate number of observations. As the sensitivity of uteri in various groups vary for acetylcholine, the comparison is only valid for each group.

TABLE 2. ISOLATED RAT UTERUS PREPARATION (relative equipotent molar ratios of 5-hydroxytryptamine and oxytocin to acetylcholine* on various tissues)

Drugs	Group 1 Anoestrus	Group 2 Induced oestrus	Group 4 Stilboestrol and reserpine-treated
Acetylcholine	100*	5	100
5-Hydroxytryptamine	42	0·5	33·0
Oxytocin	0·11	0·00015	0·06

Value of 100 has been arbitrarily fixed for acetylcholine in Group 1.

33·0 ± 3·0 (Table 1). So 5-HT was 3·0 times more potent than acetylcholine.

Oxytocin stimulated this tissue in doses ranging from 0·8 to 6·0 ng/5 ml. No tachyphylaxis was seen. The sensitivity was the same as on the anoestrous tissue and no spontaneous activity was observed. The entire dose-response curve was parallel to that of acetylcholine. Its equipotent molar ratio compared with acetylcholine was 0·06 ± 0·002 (Table 1); hence oxytocin was 1670 times more potent than acetylcholine.

The increase in sensitivity of the uterine tissue during induced oestrus (after stilboestrol treatment) to acetylcholine, 5-HT and oxytocin was 20, 86 and 730 times respectively (Table 2). The sensitivity of the tissues from the Group 4 to all the oxytocic drugs seemed to be the same as on anoestrous tissues (Tables 1 and 2).

Microscopic examination of vaginal smears. In the group in anoestrus (1), the field was full of leucocytes with a few nucleated epithelial cells, while for the induced oestrus group the field was full of cornified epithelial cells. After reserpine treatment a large number of leucocytes and a few nucleated epithelial cells were seen (Group 3). This picture was similar to dioestrus. Stilboestrol-treated tissue after reserpinization had a large number of leucocytes, a few nucleated epithelial cells, and occasional stratified epithelial cells. This resembled the dioestrous phase except for the presence of occasional epithelial cells.

Macroscopic examination of uteri. In the anoestrus group the uterine horns were 4–5 cm long, pale and quiescent. No fluid was detected inside the lumen, which was slit-like. The uterine horns of the induced oestrus group were 5–6 cm long, congested and swollen with fluid. After reserpine treatment, with and without stilboestrol, the size of the uterine horns were the same as in anoestrus, but were hyperaemic. No fluid was detected inside the lumen.

Discussion

The results with 5-HT on oestrous tissue are qualitatively similar to those of Erspamer & Correale (1953) obtained under similar circumstances. The work also gives quantitatively the comparative effects of 5-HT and oxytocin to acetylcholine on the three types of rat isolated uteri preparations. The sensitivity of tissues in induced oestrus to acetylcholine, 5-HT and oxytocin is 20, 86 and 730 times higher than in the anoestrous tissues.

The findings for the stilboestrol-treated reserpinized rat uteri are most interesting. One would expect an increase in the sensitivity to 5-HT, because smooth muscle of atria, from reserpinized rabbit, were found to have become more sensitive to 5-HT (Trendelenburg, 1960). But on the contrary there was a marked reduction in sensitivity to a level as low as in anoestrous tissue, not only to 5-HT but to the other oxytocic drugs used. This means that diethylstilboestrol failed to produce its sensitizing effects on the uteri in animals already treated with reserpine.

Could progesterone antagonize the action of diethylstilboestrol? Knaus (1927, cited by Henry & others, 1950) observed that addition of

progesterone to the bath decreased the sensitivity of the rabbit isolated uteri to posterior pituitary extract. Marshall (1962) demonstrated that the action potentials set up by oxytocin in progesterone-dominated rat uteri were infrequent, irregular and smaller in amplitude.

Vaginal smear examination of reserpinized animals, in both groups receiving stilboestrol and untreated animals, showed the presence of a large number of leucocytes and a few nucleated epithelial cells. This picture is similar to that of anoestrous phase but animals in the last group showed in addition desquamated epithelial cells. This shows that reserpine therapy has prevented the changes produced by stilboestrol on vaginal smear.

Nikitovitch-Winer & Everett (1958) suggested that reserpine increases secretion of luteotrophic hormone from the adenohypophysis, due to depressive effect of reserpine on the hypothalamus. Normally the hypothalamus has an inhibitory control over the secretion of luteotrophic hormone from the anterior pituitary, and progesterone is secreted from the corpora lutea under influence of luteotrophic hormone. Both during pregnancy and pseudopregnancy corpora lutea persist under the action of increased amount of luteotrophic hormone and secrete more progesterone. Fajer & Barraclough (1966) have produced pseudopregnancy in rats by giving reserpine, 1 mg/kg, during pro-oestrus. They measured the progesterone level in ovarian venous blood and showed a marked increase in its level after reserpine. On the basis of the work of Nikitovitch-Winer & Everett (1958), Barraclough & Saywer (1959) and Fajer & Barraclough (1966), we suggest that there is a possibility that in our work reserpine has made large amounts of progesterone available in the body of the animal. This progesterone has then antagonized the action of diethylstilboestrol on the myometrium of the reserpinized animals. This view has yet to be proved.

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