EFFECT OF PROGESTERONE THERAPY ON THE RESPONSES OF THE ISOLATED RAT STOMACH FUNDUS STRIP PREPARATIONS TO 5-HYDROXYTRYPTA-MINE AND ACETYLCHOLINE

Pages with reference to book, From 84 To 88

A. Qayum (*Present address:- PCSIR Laboratories, Peshawar.)
S.M. Yusuf (Department of Pharmacology, Army Medical College, Rawalpindi.)

Abstract

The work deals with the study of the effect of Progesterone therapy in female (virgin) albino rats, on the responses of the isolated stomach fundus strip preparations to 5-hydroxytryptamine and acetylcholine. Progesterone was injected subcutaneously in 20 mg/kg doses, daily for 14 days. 5-Hydro-xytryptamine and acetylcholine were used in the dose range of 0.2-1.6 and 2-16 ng/ml, respectively. The evaluation of data revealed that progesterone therapy did not modify the effects of 5-hydroxytryptamine and acetylcholine on this preparation (JPMA 31:84, 1981).

Introduction

Besides modifying the effects of several drugs on myometrium (Kameswaren et al., 1962; Tothill, 1967; Khan and Ahmad, 1969; Butterworth and Randall, 1970; Spilman, 1974) Progesterone has been shown to modify the effects of various pharmacologically active substances on some other systems and tissues also. Thus it has been reported that progesterone modifies the effects of oxytocin on the blood pressure of anaesthetized rat (Lloyd, 1959; Hettiaratchi and Pickford, 1968), 5-Hydroxytry-ptamine on the isolated preparations of the external ear artery of the rabbit (Fozard and Schneiden, 1970), adrenaline on the isolated rat ureter (Raz et al., 1972), and noradrenaline and isoprenaline on the urethra of anaesthetized dog (Raz et al., 1973). The present work has been designed to study the effect of progesterone therapy in rats on the responses of isolated stomach fundus strip preparations to 5-hydroxytryptamine and acetylcholine.

Material and Methods

Selection of Animals: Experiments were performed on virgin female albino rats of Sprague Dawley strain weighing 250-300G, and reared at the animal house of the Jinnah Postgraduate Medical Centre, Karachi. Progesterone was injected subcutaneously into the thighs in 20 mg/kg doses daily for 14 days (Fozard, 1971). The rats in the control group received equivalent volumes of ethyloleate in the same way as progesterone.

Preparation of Isolated Fundus Strip (Fig. 1):

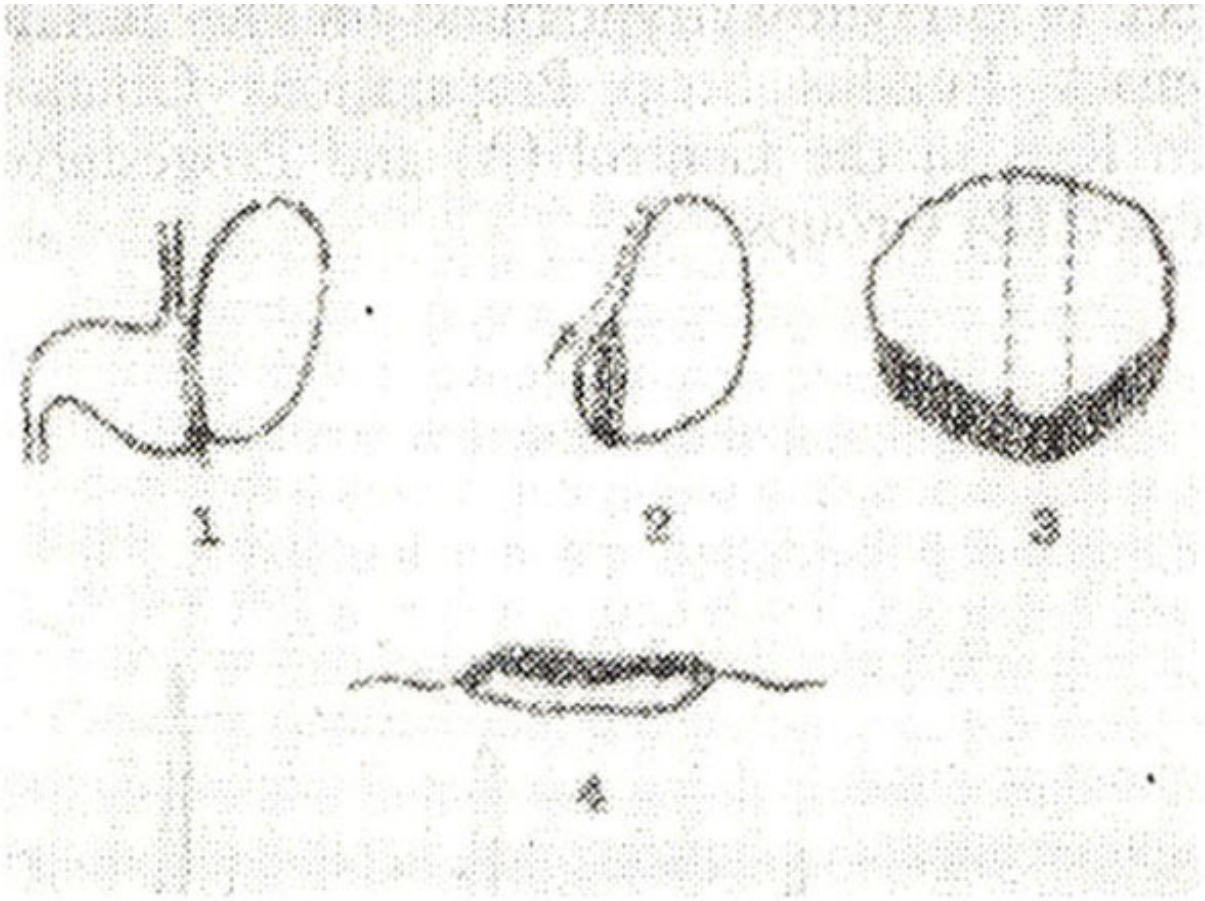

Fig. 1 : Preparation of Rat Stomach Fundus Strip.

The strip was prepared according to the method described by Khan and Anwar (1970). The stomach was removed and transferred to a petri dish containing oxygenated Tyrode solution. It was then cut along the dividing line between the fundus and pylorus. The fundus was incised along the lesser curvature and was converted into a flat piece. The margins of this piece were trimmed away and a 2 to 2.5 cm long and 3 to 4 mm wide piece was obtained from its central portion. This piece was suspended in an isolated organ bath of 10 ml capacity (Fig. 2)

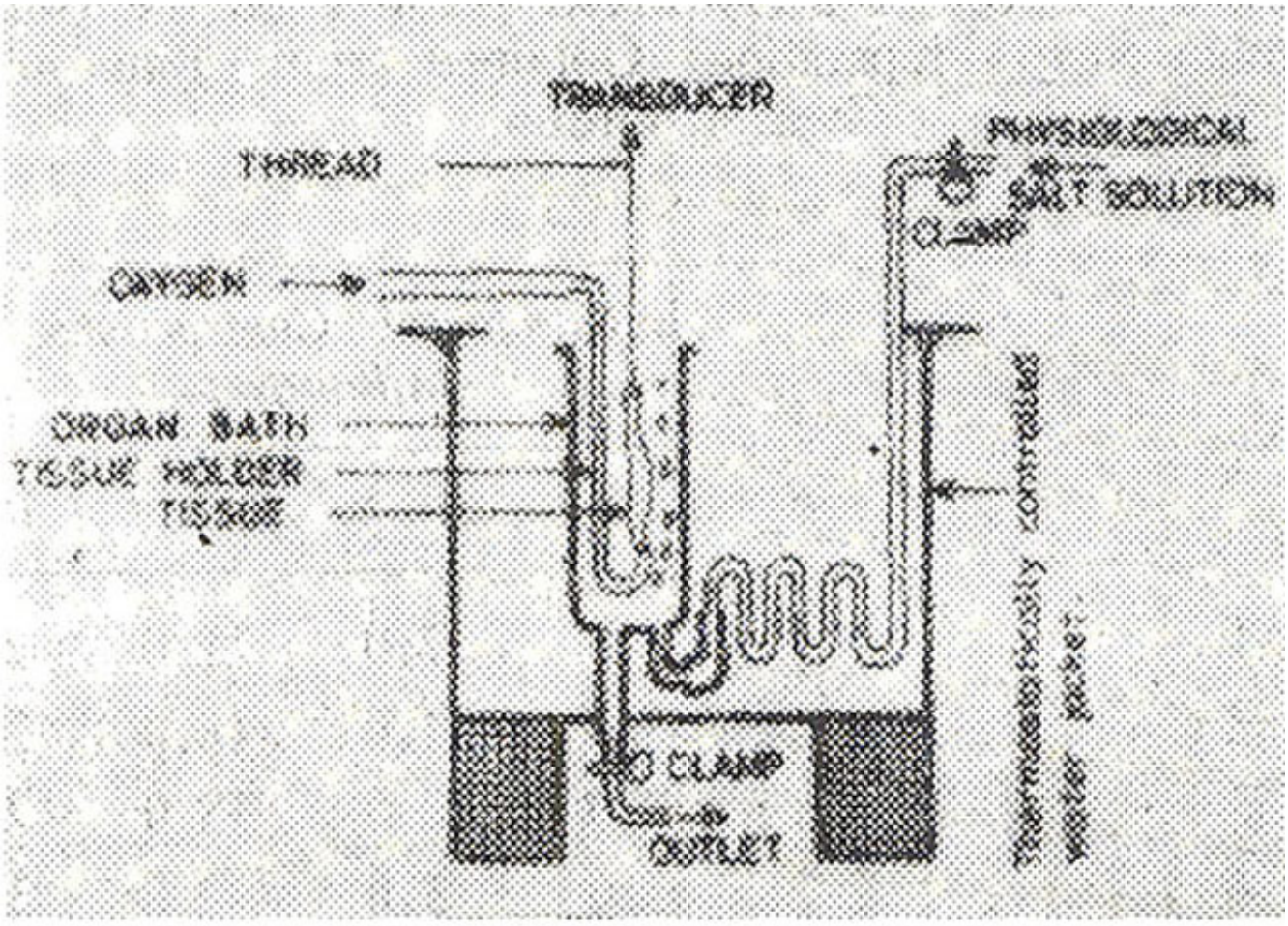

Fig. 2: Isolated Organ Bath.

containing oxygenated Tyrode solution at 37°C, and was allowed to remain in the bath for 45 to 60 minutes before starting treatment with drugs. The preparation was washed repeatedly during this period, at the end of which it was subjected to a tension of 0.5G. 5-hydroxytryptamine and acetylcholine were used in concentrations ranging from0.2-1.6 and 2.16 ng/ml, respectively. Each dose was allowed to remain in the bath for one minute. Contractions were recorded a Grass polygraph (model 7B) through a force displacement transducer (model FT03C).

(i) Stomach as a whole.

(ii) Isolated fundus along with a thin rim of pylorus. This portion was incised along dotted line.

(iii) Fundus converted into a flat plate.

(iv) The strip obtained by cutting the flat plateat dotted lines.

Nutrient Solution: Tyrode solution of the following concentration (in G/L of distilled water) was used:- NaCl, 8.0; KCl, 0.2; $CaCl_2$, 0.2; $MgCl_2$,0.1; NaH_2PO_4,0.05; $NaHCO_3$) 1.0;and glucose, 1.0. Hyoscine hydrobromide (100 ng/ml) was added to this solution in the case of experiments with 5-hydroxytryptamine (Khan, 1959).

Drugs Used: The following drugs were used ".-Acetylcholine chloride (E. . Merck), 5-hydroxytryptamine creatinine sulphate (Sigma) and progesterone (Geofman). The concentrations of acetylcholine and 5-hydroxytryptamine have been expressed in terms of active base.

Results

A. 5-HYDROXYTRYPTAMINE:

5-Hydroxytryptamine was used in concentrations of 0.2, 0.4, 0.8 and 1.6 ng/ml.

Fig. 3: Effect of 5-Hydroxytryptamine (0.2-1.6 ng/ml) on Isolated Rat Stomach Fundus Strip Preparation.

Figure 3 shows a typical tracing depicting the contractile responses to these concentrations. The salient features of the effects are described as follows:

(a) Control Group:

In a series of 8 experiments with 0.2, 0.4, 0.8 and 1.6 ng/ml doses, the mean values of the amplitudes of the 5-hydroxytryptamine induced contractions were 7.1±1.5, 15.0±2.7, 20.5± 3.1 and 26.6±2.7 mm respectively (Table I).

Table I: Effect of 5-Hydroxytryptamine on Isolated Fundus Strip Preparations Obtained from Rats in the Control (A) and Progesterone Treated (B) Groups.

Dose (*ng/ml*)	*Amplitude of Contractions* (*millimeters*)		*P*
	A	*B*	
0.2	7.1±1.5	5.5±0.7	>0.2
0.4	15.0±2.7	12.9±1.6	>0.5
0.8	20.5±3.1	18.2±2.0	>0.5
1.6	26.6±2.7	23.4±1.8	>0.2

Each value represents the mean±standard error. Figures in parentheses indicate the number of experiments.

The variations in responses ranged from 2 to 14, 5 to 28, 11 to 38 and 16 to 40 mm respectively (Table II).

Table II: Effect of 5-Hydroxytryptamine on the Isolated Fundus Strip Preparations Obtained from Rats in the Control Group.

S. No.	*Amplitude of Contractions in Millimeters*			
	0.2 *ng*	0.4 *ng*	0.8 *ng*	1.6 *ng*
1	3.0	7.0	11.0	22.0
2	5.0	14.0	18.0	20.0
3	8.0	12.0	18.0	25.0
4	2.0	5.0	12.0	16.0
5	6.0	17.0	19.0	29.0
6	7.0	15.0	21.0	30.0
7	12.0	22.0	27.0	31.0
8	14.0	28.0	38.0	40.0

(b) Progesterone Treated Group:

In a series of 9 experiments with 0.2, 0.4, 0.8 and 1.6 ng/ml doses, the mean values of the amplitudes of contractions were 5.5±0.7, 12.9±1,6, 18.2±2.0 and 23.4±1.8 mm respectively (Table I). The variations in responses ranged from 2 to 8, 4 to 21, 8 to 29 and 16 to 34 mm respectively (Table III).

Table III: Effect of 5-Hydroxytryptamine on the Isolated Fundus Strip Preparations Obtained from Progesterone Treated Rats.

S. No.	Amplitude of Contractions in Millimeters			
	0.2 *ng*	0.4 *ng*	0.8 *ng*	1.6 *ng*
1	4.0	13.0	23.0	27.0
2	6.0	15.0	20.0	24.0
3	8.0	12.0	16.0	21.0
4	7.0	13.0	17.0	20.5
5	7.0	17.0	21.0	25.0
6	5.0	13.0	18.0	26.0
7	2.0	4.0	8.0	16.0
8	3.0	8.0	12.0	17.0
9	8.0	21.0	29.0	34.0

The differences in the amplitudes of contractions in the above mentioned two groups were statistically not significant (Table I). The dose response curves for both the groups are shown in Fig. 4.

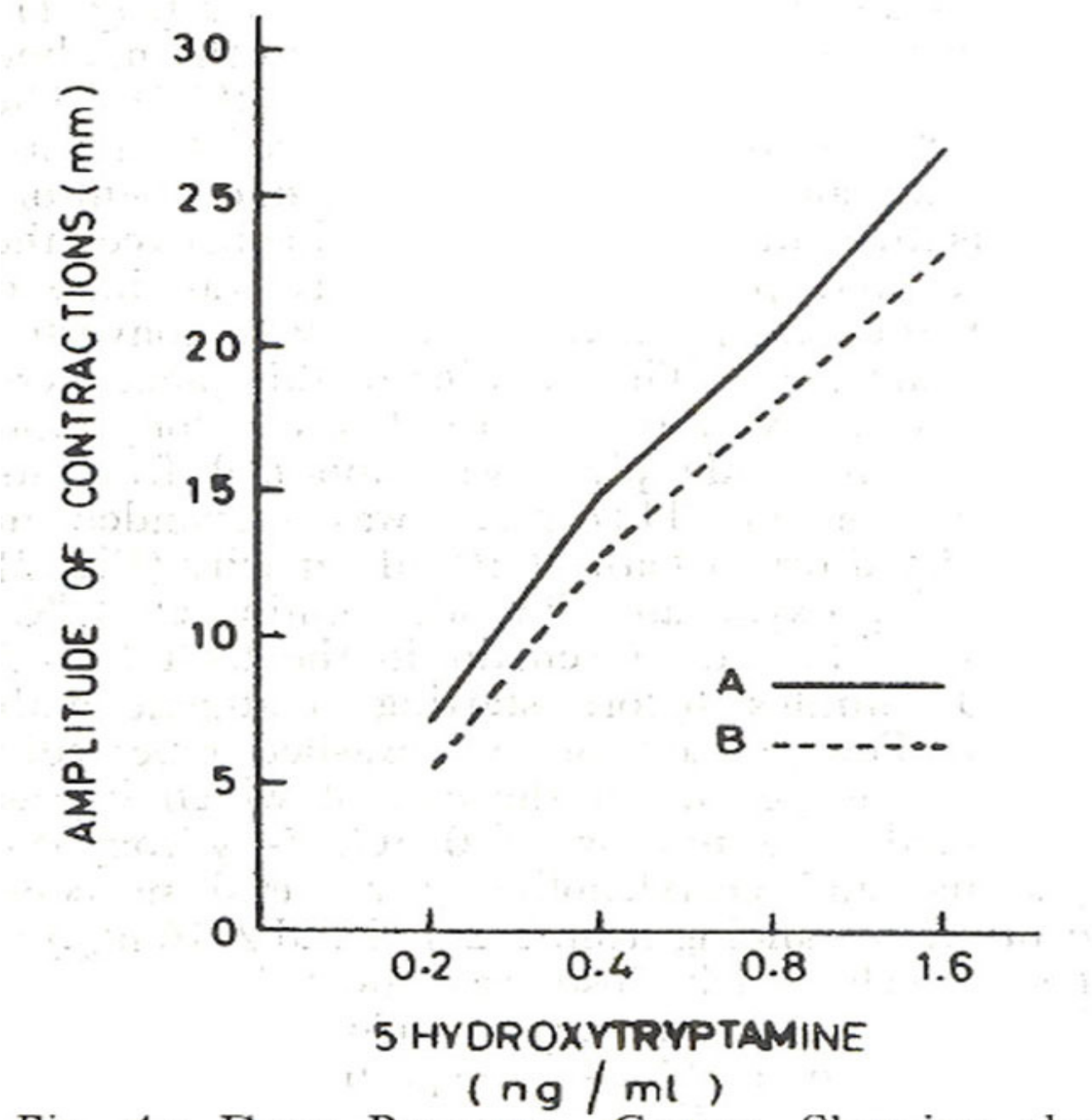

Fig. 4: Dose Response Curves Showing the Effect of 5-Hydroxytryptamine on the Isolated Stomach Fundus Strip Preparations Obtained from Rats in the Control (A) and Progesterone Treated (B) Groups.

B. ACETYLCHOLINE

Acetylcholine was used in concentrations of 2, 4, 8 and 16 ng/ml.

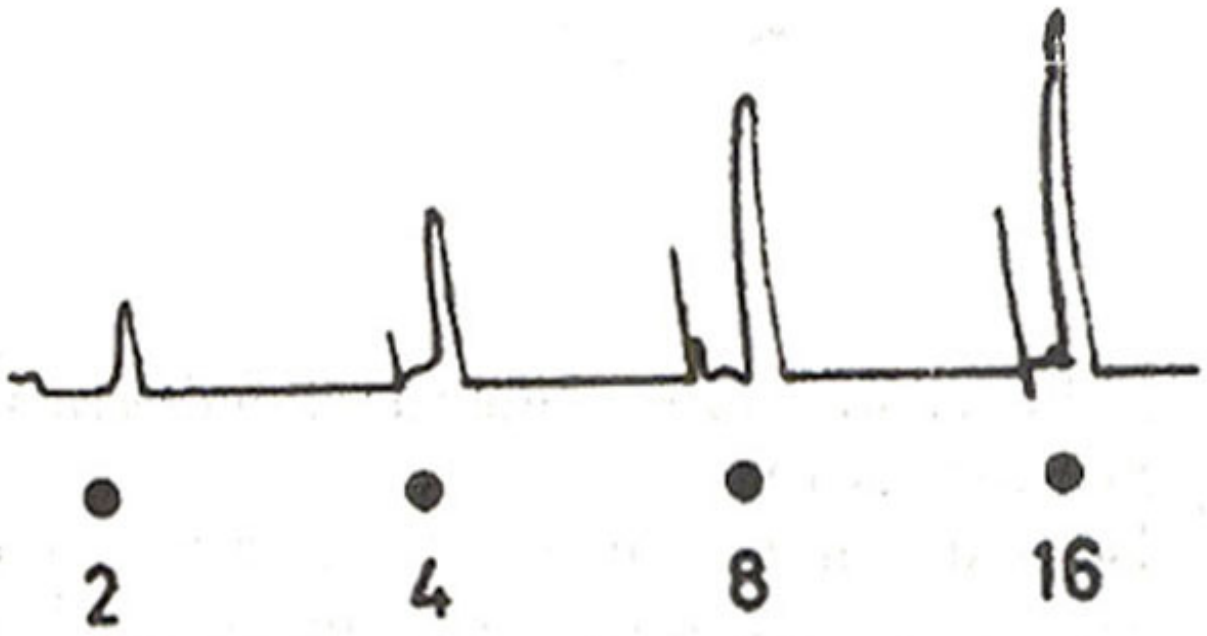

Fig. 5. Effect of Acetylcholine (2-16 ng/ml) on the Isolated Rat Stomach Fundus Strip Preparation.

Figure 5 shows a typical tracing depicting contractile responses to these concentrations. The main features of the effects are described below :-

(a) Control Group :

In a series of 11 experiments with 2, 4, 8 and 16 ng/ml doses, the mean values of the amplitudes were4.6±0.8,10.4±1.4,16.0±1 -8 and 21.3±2.2 mm respectively (Table IV).

Table IV: Effect of Acetylcholine on Isolated Fundus Strip Preparations Obtained from Rats in the Control (A) and Progesterone Treated (B) Groups.

Dose ng/ml	*Amplitude of Contractions (millimeters)*		*P*
	A	*B*	
2 ..	.. 4.6±0.8	5.1±0.8	>0.5
4 ..	.. 10.4±1.4	9.1±1.4	>0.5
8 ..	.. 16.0±1.8	15.0±1.4	>0.5
16 ..	.. 21.3±2.2	21.9±1.8	>0.5
	(11)	(9)	

The variations in these responses ranged from 1 to 8,4 to 18, 7 to 24 and 8 to 33 mm respectively Table

V).

Table V: Effect of Acetylcholine on the Isolated Fundus Strip Preparations Obtained from Rats in the Control Group.

S. No.	Amplitude of Contractions in Millimeters			
	2 *ng*	4 *ng*	8 *ng*	16 *ng*
1	8.0	18.0	22.0	33.0
2	8.0	13.0	18.0	27.0
3	4.0	10.0	14.0	21.0
4	7.5	14.0	20.0	25.0
5	6.5	15.0	24.0	27.0
6	2.0	4.0	7.0	8.0
7	4.0	10.0	14.0	22.0
8	1.0	8.0	14.0	16.0
9	7.0	14.0	24.0	25.0
10	1.0	4.0	7.0	12.0
11	2.0	5.5	12.0	18.0

(b) Progesterone Treated Group :

In a series of 9 experiments Avith 2, 4, 8 and 16 ng/ml doses, the mean values of the amplitudes of contractions were 5.1 ±0.8, 1.4, 15.0±1.4, and 21.9±1 - 8 mm respectively (Table IV).

Table VI: Effect of Acetylcholine on the Isolated Fundus Strip Preparations Obtained from Progesterone Treated Rats.

S. No.	Amplitude of Contractions in Millimeters			
	2 *ng*	4 *ng*	8 *ng*	16 *ng*
1	6.0	9.0	15.0	20.0
2	5.0	10.0	17.0	22.5
3	3.0	6.0	15.0	30.0
4	10.0	18.0	24.0	26.0
5	8.0	12.0	17.0	30.0
6	5.0	7.0	10.0	15.0
7	3.0	6.0	13.0	17.0
8	2.0	4.0	10.0	18.0
9	4.0	10.0	14.0	19.0

The variations in these responses ranged from 2 to 10, 4 to 18, 10 to 24 and 15 to 30 mm respectively (Table VI).

The differences in the amplitudes of contractions in the above mentioned two groups were statistically not significant (Table IV). The dose response curves for both the groups are shown in Fig. 6.

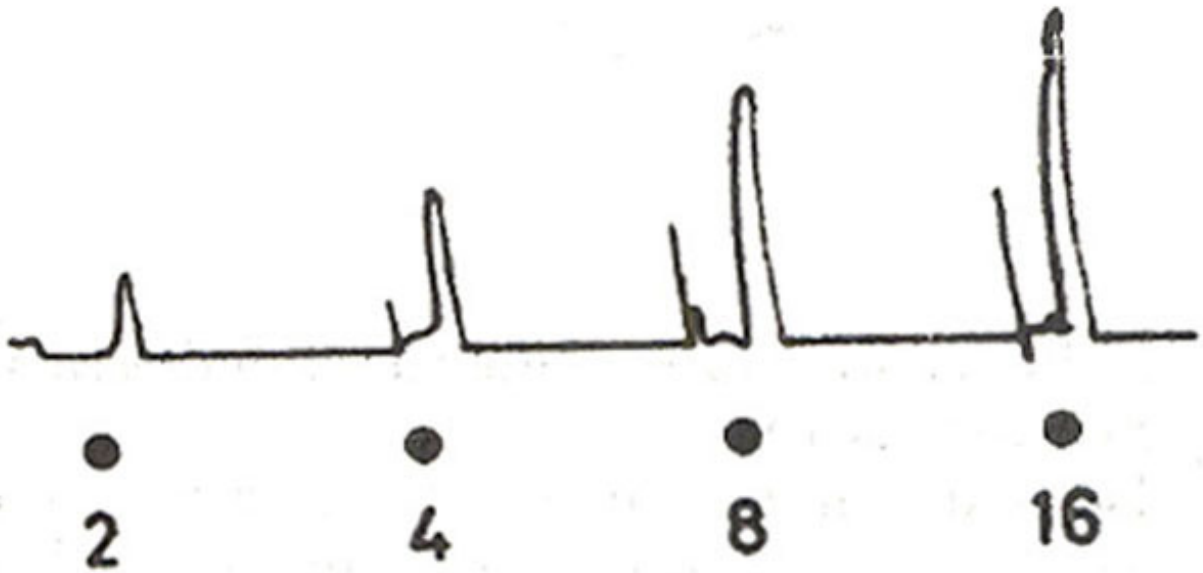

Fig. 5. Effect of Acetylcholine (2-16 ng/ml) on the Isolated Rat Stomach Fundus Strip Preparation.

Discussion

The evaluation of data indicates that, when administered subcutaneously in 20 mg/kg daily doses, progesterone does not significantly modify the amplitude of contractile responses of the isolated fundus strip preparations to 5-hydroxy-tryptamine and acetylcholine. As progesterone therapy has been found to decrease the sensitivity of the rat uterus to acetylcholine and 5-hydroxy-tryptamine (Khan and Ahmad, 1969), these observations show that the smooth muscle of the rat stomach fundus is not affected by progesterone in a way similar to the myometrium. In this respect the smooth muscle of the rat fundus also differs from the smooth muscle of the central ear artery of rabbit, which depicted an increase in sensitivity to 5-hydroxytryptamine after the animals were treated with progesterone (Fozard and Schneiden, 1970).

References

1. Butterworth, K.R. and Randall, MJ. (1970) The effects of alpha and beta adrenoceptor blocking agents on the responses of the rat uterus to catecholamines throughout the oestrus cycle. Br. J. Pharmacol., 40:160.
2. Fozard, J.R. (1971) Effects of progesterone on the cardiovascular responses to amines and to sympathetic stimulation in the pithed rat. Br. I. Pharmacol., 41:187.
3. Fozard, J.R. and Schneiden, H. Steroidal hormones and vascular reactivity in the rabbit. III. Third International Migraine Symposium, edited by Cochrane, A.L.A. London, Heinemann, 1970.
4. Hettiaratchi, E.S.G. and Pickford, M. (1968) The effect of oestrogen and progesterone on the pressor action of angiotensin* in the rat. J. Physiol. Lond., 196:447.
5. Kameswaren, L., Pennefather, J.N. and West, G.B. (1962) Possible role of histamine in rat pregnancy. J. Physiol. Lond., 164:139.
6. Khan, I. Drugs modifying the action of 5-hydroxytrypta-mine. Edinburgh, University of Edinburgh, 1959 (Ph.D. Thesi).
7. Khan, I. and Ahmad, N. (1969) Effects of progesterone therapy on the stillboestrol induced sensitivity of isolated rat uterus preparations. Br. J. Pharmacol., 35:332.
8. Khan, I. and Anwar, M. (1970) A study of adrenergic receptors in isolated fundus (stomach) strip preparation. Medicus, 40:243.
9. Lloyd, S. (1959) The vascular responses of the rat during reproductive cycle. J. Physiol. Lond., 148:625.
10. Raz, S., Zeigler, M. and Caine, M. (1972) Hormonal influence on the adrenergic receptors of the ureter. Br. J. Urol., 44:405.
11. Raz, S., Zeigler, M. and Caine, M. (1973) The effect of progesterone on the adrenergic receptors of the urethra. Br. J. Urol., 45:131.
12. Spilman, C.H. (1974) Oviduct response to prostaglandins; influence of estradiol and progesterone. Prostaglandins, 7:465.
13. Tothill, A. (1967) Investigation of adrenaline reversal in the rat uterus by the induction of resistance to isoprenaline. Br.J. Pharmacol., 29:291.