Br. J. Pharmac. Chemother. (1968), 32, 322–329.

PHARMACODYNAMIC ACTION OF CYCLOHEXIMIDE ON THE RAT UTERUS

BY

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(*Received September* 14, 1967)

Treatment of rats with oestrogen is followed within 2 min by the transcription of deoxyribonucleic acid in the uterus (Means & Hamilton, 1966), which leads to the synthesis of new protein. In previous work with the rat uterus (Tothill, 1967a, b) results were obtained which suggested that the response of the uterus to some drugs, in particular the catecholamines, varied in accordance with the functional state of the organ, and the difference in response could be induced by raising oestrogen levels. Thus, as oestrogen stimulates the synthesis of new protein, it seemed of interest to test the effects of inhibitors of protein synthesis on the rat uterus to see whether they had any effect on the pharmacological behaviour of the organ. Cycloheximide (Actidione) was selected for this purpose; it is an antibiotic produced by *Streptomyces griseus* (Whiffen, Bohonos & Emerson, 1946), which inhibits protein synthesis in yeasts and fungi and in intact rats (Young, Robinson & Sacktor, 1963; Colombo, Felicetti & Baglioni, 1965; Gorski & Axman, 1964). Wettstein, Noll & Penman (1964) showed that this substance inhibits protein synthesis at a late stage by hindering the movement of ribosomes along messenger ribonucleic acid. It blocks polypeptide chain formation by inhibiting the read-out process and thus inhibits the multiplication of numerous viruses (Haff, 1964; Küchler, Küchler & Bradler, 1965).

METHODS

Animals

The experiments were carried out on thirty-seven Wistar female albino rats weighing 218–225 g. Four groups were set up.

a. Induced oestrus (twelve rats). Diethystilboestrol in arachis oil was injected subcutaneously in a dose of 2.5 mg/kg 40–45 hr before the experiment was carried out.

b. Dioestrus (twelve rats).

c. Induced oestrus, reserpinized (nine rats). Treated with stilboestrol as in group *a* but also injected subcutaneously with reserpine in a dose of 7.5 mg/kg once daily for 2 days.

d. Dioestrus, reserpinized as in group *c* (four rats).

On the day of the experiment the degree of cornification of the vaginal epithelium was assessed by examining a vaginal smear, in order to confirm that the uterus was in the desired functional state. The animal was killed by a blow on the head and the main vessels of the neck were cut. The uterus was removed and each horn was suspended separately in a 25 ml. organ bath containing mammalian

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Ringer-Locke at 36.5° C. Recordings were made on a smoked drum with an isotonic lever with frontal writing point, adjusted to have a magnification of 2.5 and a constant load of 2.0 g. Intrinsic rhythm usually appeared within 5–10 min, and preparations in which this was not the case were discarded. Experiments were carried out on each horn and lasted for up to 3 hr.

Drugs

The following drugs were used. Cycloheximide (β-[2(3,5-dimethyl-2-oxo*cyclo*hexyl)-2-hydroxyethyl] glutarimide, Sigma Chemical Company); two samples were used, with melting points of 110° C and 106° C, respectively, and the pharmacological effects were identical. Adrenaline acid tartrate (Burroughs Wellcome). Tyramine (L. Light and Co., Ltd.). Reserpine (Serpasil, Ciba Laboratories Ltd.); 80 mg was dissolved in a few drops of glacial acetic acid, and to this solution were added propylene glylcol 0.8 ml., ethanol 0.8 ml. and distilled water 18 ml. Diethylstilboestrol (Organon); 50 mg was dissolved in 1 ml. of ethanol. The solution was emulsified with 50 ml. of arachis oil and then allowed to stand overnight at 50° C to evaporate off the ethanol.

RESULTS

Effect of cycloheximide on the uterus in induced oestrus (group a)

Addition of cycloheximide to a concentration of 0.1–5.0 μg/ml. in the bath completely inhibited the intrinsic rhythm of the uterus in induced oestrus (Fig. 1). Inhibition was complete for an average period of 6 min; the uterus then began to contract again. The initial contractions were usually about 10–20% of the height of the original intrinsic contractions, but within 6–15 min they had returned to the normal size. At this juncture

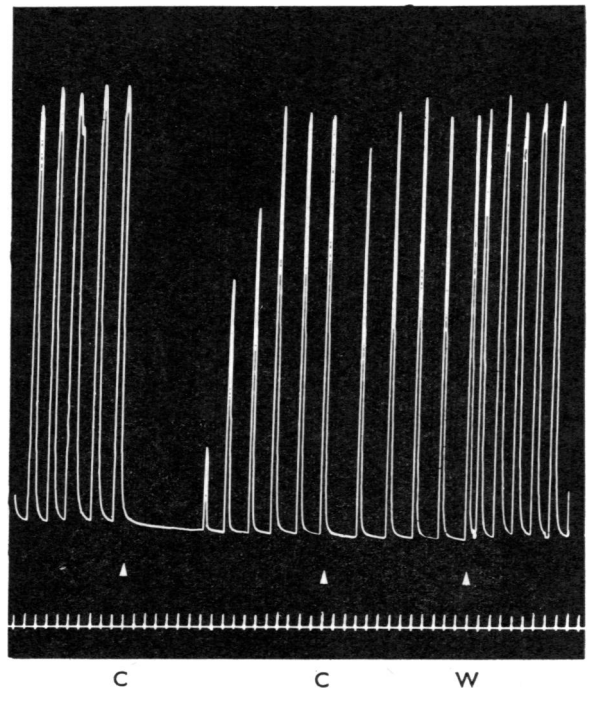

Fig. 1. Uterus in induced oestrus. C, cycloheximide 100 ng/ml.; W, wash. Note that the second dose of cycloheximide produced only slight inhibition. Time marks, 1 min.

addition of the same dose of cycloheximide had little effect. In a similar experiment the drug was left in contact with the uterus and recovery was allowed to occur ; the bath fluid was then placed on a fresh preparation and caused complete inhibition (Fig. 2). Recovery from inhibition therefore could not have resulted from any substantial degree of decomposition of the drug. The uterus thus escapes from cycloheximide and becomes resistant to it. If, during the period of recovery, the bath fluid was changed, however, the original rhythm reappeared immediately (Fig. 1).

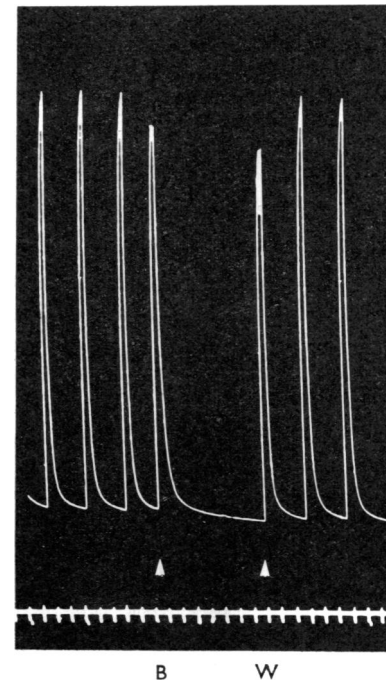

Fig. 2. Uterus in induced oestrus. B: bath fluid from a different preparation to which cycloheximide (100 ng/ml.) had been added and resistance had occurred. W: wash. Time marks, 1 min.

Because low doses of cycloheximide seemed to have a definite pharmacological effect on the rat uterus in induced oestrus, attempts were made to obtain a dose-response curve. These were unsuccessful on all occasions, because a second similar dose of the drug applied after the first had been washed off produced less inhibition, or even slight or no inhibition (Fig. 3). It therefore seemed that cycloheximide could act maximally only once on a given preparation. It was considered that this anomalous effect could be explained if cycloheximide were exerting its inhibitory effect by discharging stores of catecholamines, because in these circumstances its effect would not be repeatable until the stores had become repleted.

Effect of cycloheximide on the dioestrous uterus (group b)

Dioestrous uteri, in which catecholamine stores are low (Wurtman, Chu & Axelrod, 1963), failed to respond to doses of tyramine 1 μg/ml. (Fig. 4). Cycloheximide in doses of 0.5–1.0 g/ml. was ineffective and doses of 100 μg/ml. still had no effect (Fig. 5).

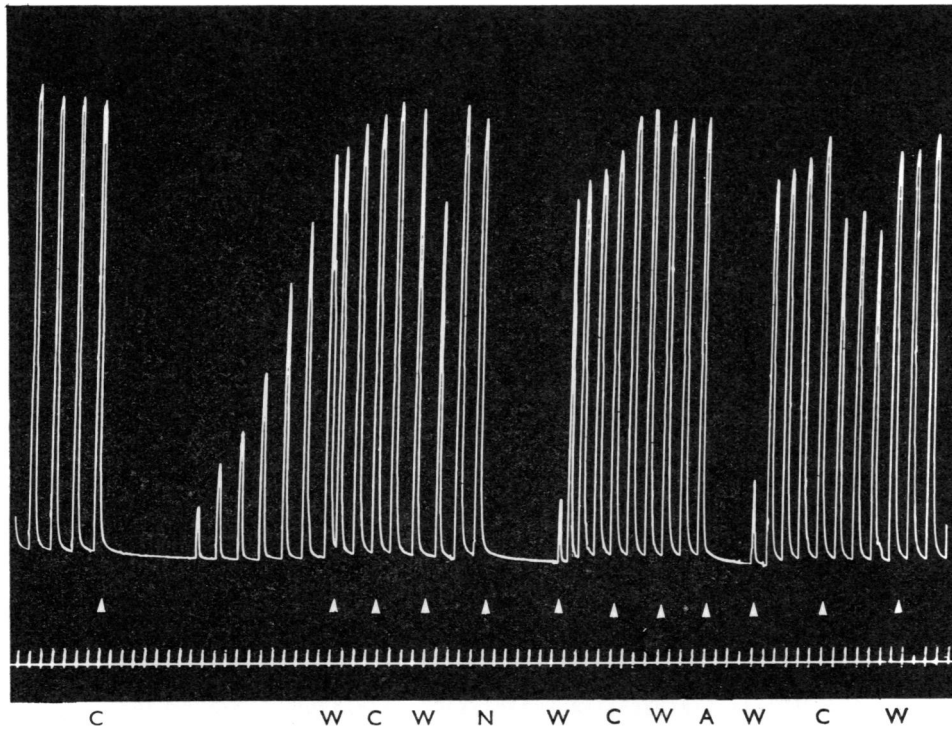

Fig. 3. Uterus in induced oestrus. C, cycloheximide (1 μg/ml.); W, wash; N, noradrenaline (1 μg/ml.); A, adrenaline (100 ng/ml.). Note that adrenaline seems to restore slightly the inhibitory action of cycloheximide. Time marks, 1 min.

Higher doses (400 μg/ml.) did induce some inhibition but the preparation did not recover its original rhythm after washing, and the inhibition may therefore have been caused by toxicity.

Investigation of the possible release of catecholamines from stores by cycloheximide

Tyramine has been shown to act by releasing catecholamines from stores (Burn & Rand, 1958) and can therefore be used as a reagent for detecting the presence of catecholamines in stores. Tyramine 1 μg/ml. caused complete inhibition of the uterus in induced oestrus (Fig. 4). On the same preparation, after washing, a second dose of tyramine was less effective, indicating progressive emptying of the catecholamine stores. In view of this, cycloheximide was used on the second horn of the uterus from the same animal, in which the stores were still intact. It was first shown that the addition of tyramine in a concentration of 1 μg/ml. inhibited the intrinsic rhythm of the uterus. On the same preparation cycloheximide in a dose of 100 ng/ml. was ineffective. A dose of 20 μg/ml. caused inhibition, and a second similar dose had no effect. The preparation was then washed, and the addition of tyramine in a dose of 1 μg/ml. was now almost ineffective (Fig. 6).

These observations were therefore consistent with the hypothesis that cycloheximide exerts its action by depleting the stores of catecholamines. An alternative explanation

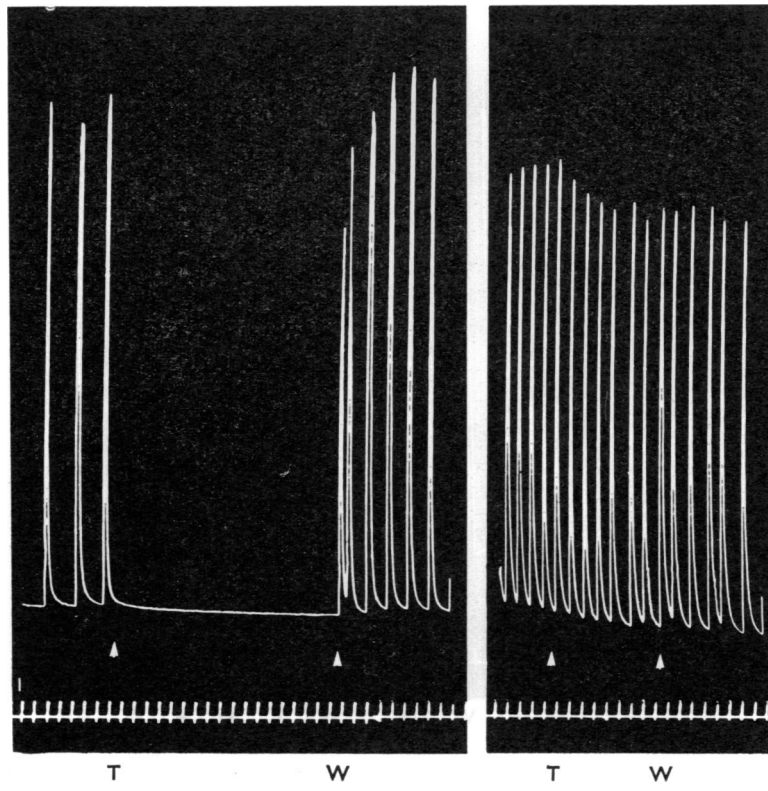

Fig. 4. Left, uterus in induced oestrus; right, dioestrous uterus. In both instances, T, tyramine (1 μg/ml.); W, wash. Time marks, 1 min.

would be that cycloheximide itself stimulates β-adrenoceptive receptors. To investigate this possibility the experiment illustrated in Fig. 3 was carried further. After the induction of resistance to cycloheximide the uterus was washed; addition of noradrenaline (1 μg/ml.) caused inhibition; the uterus was therefore still able to respond to β-receptor stimulation. The uterus was washed and cycloheximide (1 μg/ml.) was added; no inhibition occurred. Adrenaline was then added in a concentration of 100 ng/ml., and caused complete inhibition; the uterus was therefore still fully responsive to β-receptor stimulation. In view of this observation cycloheximide itself could not be a β-receptor agonist. Cycloheximide was again added in a concentration of 1 μg/ml.; a slight inhibition was produced, which could well have been the result in this instance of slight repletion of stores by the added adrenaline and their discharge by cycloheximide. In replicates of the entire experiment this inhibition was not observed again with certainty.

Effects of cycloheximide on the reserpinized uterus: induced oestrus (group c)

Tyramine (1 μg/ml.) was ineffective and therefore treatment with reserpine had been effective in depleting the stores. Cycloheximide in doses as high as 100 μg/ml. did not inhibit the intrinsic rhythm of the uterus (Fig. 5).

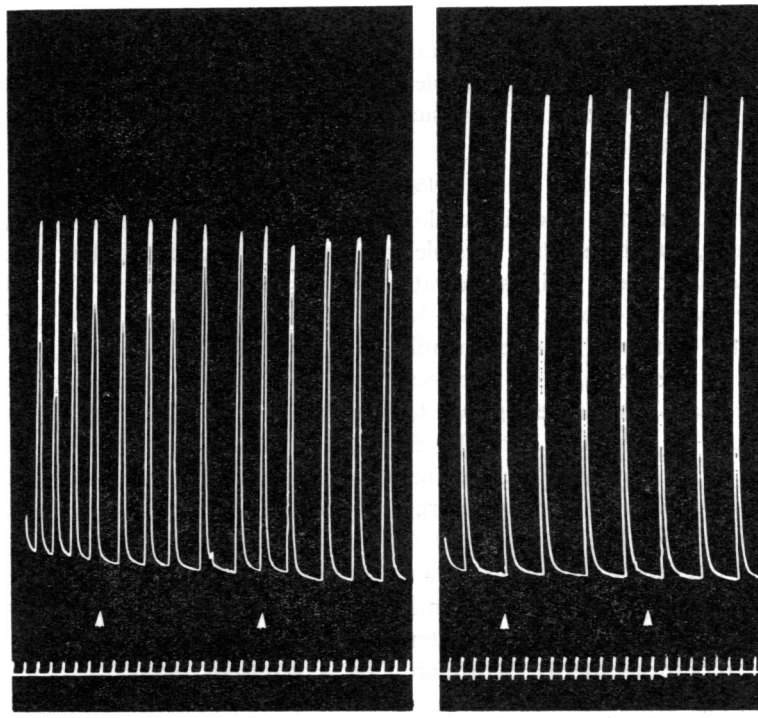

Fig. 5. Left, uterus in dioestrus; right, uterus from a rat treated with stilboestrol and reserpine. In both instances, C, cycloheximide (100 μg/ml.); W, wash. Time marks, 1 min.

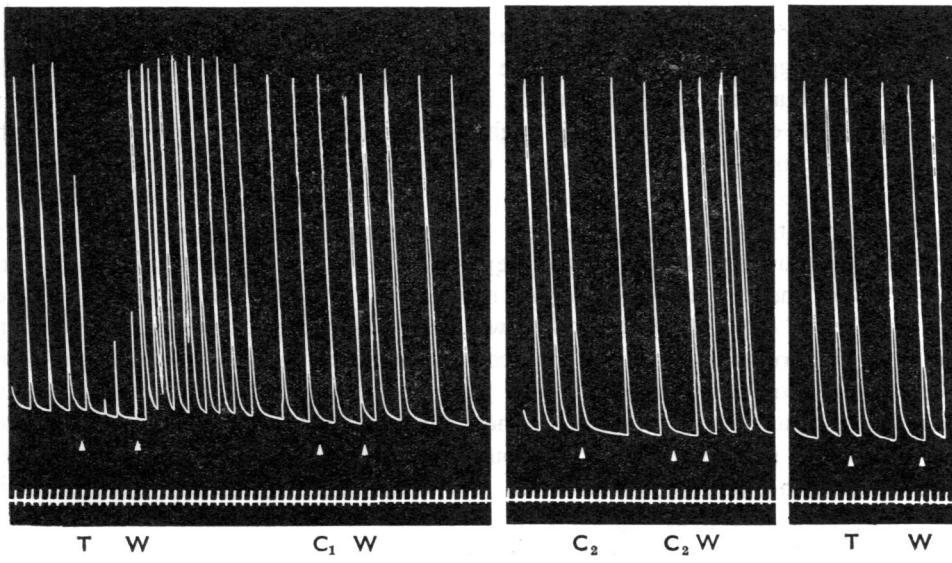

Fig. 6. Uterus in induced oestrus. T, tyramine (1 μg/ml.); W, wash; C_1, cycloheximide (100 ng/ml.); C_2, cycloheximide (20 μg/ml.). First gap in tracing, 45 min; second gap, 6 min. Time marks, 1 min.

Dioestrus (group d)

Both tyramine (Fig. 4) and cycloheximide in the same respective doses had no apparent effect and the stores were therefore depleted. Addition of adrenaline, noradrenaline or isoprenaline fully inhibited the intrinsic rhythm, and the uterus was therefore still sensitive to catecholamines.

DISCUSSION

The pharmacodynamic effect of cycloheximide may be the result of inhibition of protein synthesis, but three other possible mechanisms of action must first be discussed. Cycloheximide completely inhibits the rat uterus in induced oestrus. It might therefore be acting directly on β-receptors or on some other receptive substance, or it might be acting indirectly by release of catecholamines from stores. It seems unlikely that cycloheximide is a β-receptor agonist, because it fails to act in the reserpinized preparation in which the catecholamines were shown to be still effective. This supposition also seems unlikely because the structural formula of cycloheximide bears no relation to that of the catecholamines. If, however, cycloheximide were acting on some other receptive substance which had been altered by reserpine, then it should have been effective in the dioestrous uterus, but this was not the case. It therefore seems probable that cycloheximide does not inhibit the uterus by a direct mechanism.

The third possibility is that cycloheximide acts on the rat uterus by releasing catecholamines from stores. In the oestrous uterus these stores are high, but they are low or absent in the dioestrous uterus (Wurtman, Chu & Axelrod, 1963). Tyramine, which is known to release catecholamines from stores in other organs (Burn & Rand, 1958), completely inhibited the intrinsic rhythm of the uterus in induced oestrus, but was ineffective in dioestrus. It thus seems that tyramine acts in the rat uterus as it does on other organs. Cycloheximide was active in low doses in induced oestrus and was without effect, even in high doses, on the dioestrous uterus. Furthermore, it had no effect in induced oestrus when the stores had been artificially depleted by treatment with reserpine. This evidence suggests that cycloheximide releases catecholamines from stores and that it is the catecholamines which cause the inhibition. If cycloheximide inhibits the rat uterus by release of catecholamines it might be expected to have a motor effect on uteri in which the action of these substances is usually motor—for example, the pregnant cat uterus.

From the results of the present work it is concluded that cycloheximide does not act on β-receptors but that it inhibits the uterus by liberating catecholamines from stores. The nature of the catecholamine stores in the uterus has not been fully elucidated. Wurtman, Chu & Axelrod (1963), in their work on the rat uterus, suggested that there are two stores, one hormone-sensitive which contains mainly adrenaline, and a second store which takes up noradrenaline. It has not yet been established by what mechanisms the catecholamines pass into stores and are held there, but from the results of the present work it may be suggested that continuous synthesis of protein is necessary to keep catecholamines in stores, and that when this synthesis is inhibited by cycloheximide the stores become depleted.

SUMMARY

1. The pharmacodynamic action of cycloheximide, an inhibitor of protein synthesis, was studied on the isolated rat uterus in different functional states.

2. Cycloheximide in low doses inhibited the uteri from animals treated with stilboestrol but was without effect, even in high doses, on uteri from rats in dioestrus or treated with reserpine.

3. It is shown that cycloheximide does not stimulate β-receptors but seems to inhibit the uterus by liberating catecholamines from stores.

4. It is possible that continuous synthesis of protein is necessary for maintaining catecholamine stores.

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