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INVESTIGATION OF ADRENALINE REVERSAL IN THE RAT UTERUS BY THE INDUCTION OF RESISTANCE TO ISOPRENALINE

BY

ANNE TOTHILL

From the Department of Pharmacology, Guy's Hospital Medical School, London

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Ahlquist (1948) classified the rat uterus as possessing primarily β-receptors for adrenaline, but subsequently (1962) suggested that all uteri contain α-excitatory and β-inhibitory catecholamine receptors. However, Rudzik & Miller (1962) concluded that the rat uterus contains α- and β-receptors and that both produce relaxation of the uterus in response to drugs. Levy & Tozzi (1963) investigated the nature of the receptors in the rat uterus and concluded that α-receptors were absent; furthermore, they queried the existence of the α-inhibitory receptors postulated by Rudzik & Miller (1962), because inhibition of the uterus by adrenaline was not blocked by α-receptor blocking agents.

In view of the lack of agreement in these conclusions it seemed desirable to investigate whether reversal of the inhibitory action of adrenaline to a motor effect in the rat uterus is a simple response of the α-receptors to adrenaline or if some other factor is involved, such as variation in the functional state. The nature of the receptors was studied further by using blocking agents and by the method of Butterworth (1963) for modifying the action of adrenaline by preliminary treatment with isoprenaline.

METHODS

Animals

The experiments were carried out on 291 Wistar female albino rats weighing about 200 g; the following four groups were set up.

(1) *Induced oestrus* (237 rats). Diethylstilboestrol in arachis oil was injected subcutaneously in a dose of 2.5 mg/kg 40–45 hr before the experiment was carried out.

(2) *Progestational phase* (20 rats). The animals were ovariectomized by the dorsal route under ether anaesthesia. They were then injected with 5 mg progesterone in arachis oil intraperitoneally and 2.5 mg/kg stilboestrol subcutaneously; 5 mg progesterone were given intraperitoneally on each of the next five days, and the animals were used on the sixth day.

(3) *Pregnancy* (18 rats). Rats were used on the 20th–22nd day of pregnancy and in all cases before parturition had occurred.

(4) *Natural oestrus* (16 rats).

On the day of the experiment the degree of cornification of the vaginal epithelium was assessed by examining a vaginal smear. The animal was then 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 Ringer-Locke solution at 37° 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 g. Intrinsic rhythm usually appeared within 5–10 min. Experiments were carried out on each horn and lasted for up to 6 hr.

Drugs

Isoprenaline hydrogen sulphate (British Drug Houses), adrenaline acid tartrate (Burroughs Wellcome), noradrenaline bitartrate (Bayer Products), phenylephrine (Boots Pure Drug Co., Ltd.), 5-hydroxytryptamine creatinine sulphate (May and Baker, Ltd.), oxytocin (Parke Davis & Co.), phentolamine (Rogitine, Ciba), phenoxybenzamine (Smith, Kline and French), dihydroergotamine (Sandoz), hydergine (Sandoz), pronethalol (Nethalide, Imperial Chemical Industries) and bromolysergic acid (BOL, Sandoz). All dilutions were made with Ringer-Locke solution. Progesterone and diethylstilboestrol (Organon Laboratories).

RESULTS

Preparations of the uterus in induced oestrus

Response of the uterus to drugs acting on α- and β-receptors

The uterus in the phase of induced oestrus was completely inhibited by adrenaline (100 ng/ml.), and partially inhibited by noradrenaline (100 ng/ml.) and by phenylephrine (1 μg/ml.).

Isoprenaline resistance; the reversal phenomenon in induced oestrus

Addition of isoprenaline (100 ng/ml.) caused immediate total inhibition of the intrinsic rhythm. The rhythm reappeared rapidly after several washings. Repetition of the dose of isoprenaline again caused total inhibition. When the uterus was left in contact with isoprenaline without washing, it began to contract again after 7–14 min in a rhythm which differed from the intrinsic rhythm. The first contractions were small (about 1 mm); they then became larger but in no case did they exceed the height of the intrinsic rhythmic contractions. The frequency was usually slower than that of the intrinsic rhythm. Further doses of isoprenaline no longer produced inhibition (Fig. 1). The effects observed were not due to a change in the pH of the Ringer-Locke solution which, as measured on a pH meter, remained within the range of 7–7.02 over a period of 1 hr after the isoprenaline had been added. The contents of an organ-bath in which escape of contractions had occurred caused immediate total inhibition when added to a fresh preparation (Fig. 2); the escape, therefore, could not have been due to decomposition of the isoprenaline. The state of the uterus after escape from isoprenaline will be described as resistance, and treatment of the uterus with isoprenaline before exposure to other agents will be referred to as priming. Two or three changes of Ringer-Locke solution were insufficient to abolish resistance, but after five or six washes over a period of 8–12 min the addition of isoprenaline again produced inhibition. The effect of different concentrations of isoprenaline was then investigated. Resistance did not always develop with concentrations of 10 and 1 ng/ml., since a further dose of isoprenaline often produced inhibition after escape had apparently occurred. A concentration of 1 μg/ml. induced resistance, but the intrinsic rhythm did not always reappear after washing. A concentration of 100 ng/ml. was therefore used throughout.

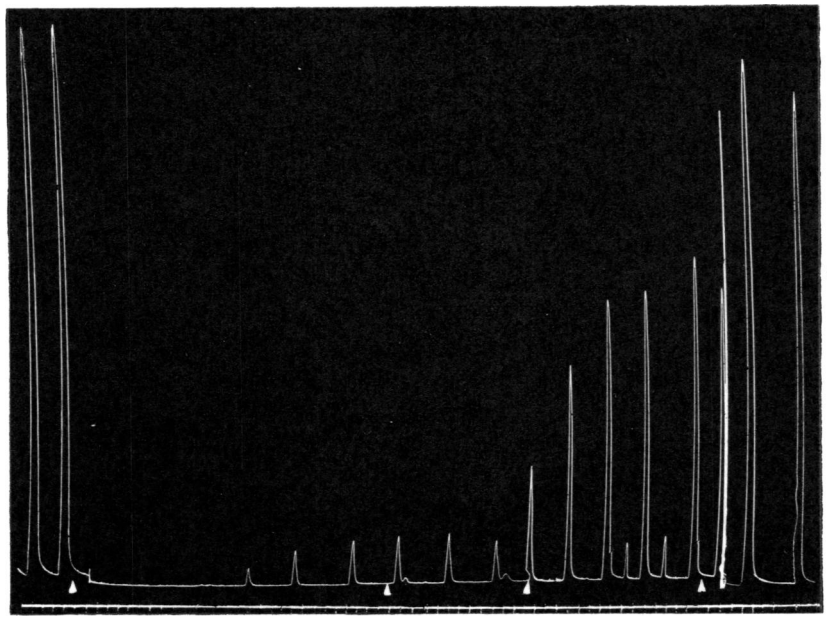

Fig. 1. Oestrous uterus. First, second and third arrows: isoprenaline, 100 ng/ml.; fourth arrow: wash. In this instance the latent period before return of sensitivity to isoprenaline was 8 min. Time marks 30 sec.

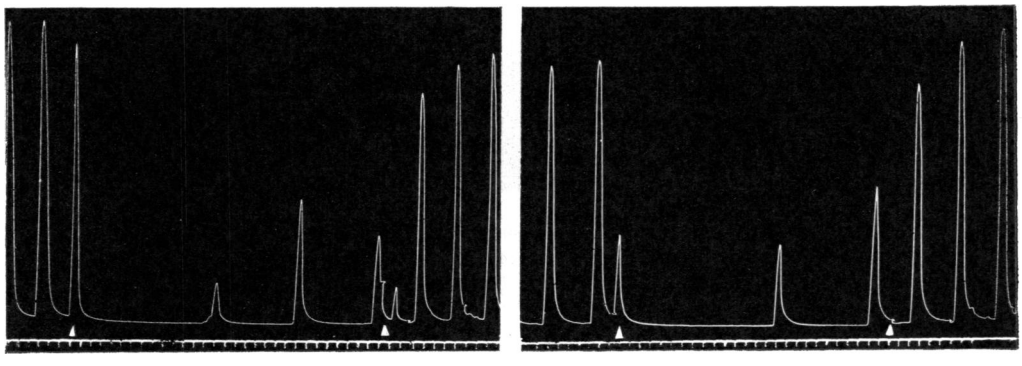

Fig. 2. Oestrous uteri. Left tracing: first arrow: isoprenaline, 100 ng/ml.; second arrow: bath fluid drained. Right tracing: first arrow: bath fluid from the first preparation put into an organ bath containing a fresh preparation; second arrow: wash. Time marks 30 sec.

When adrenaline, noradrenaline and phenylephrine in the concentrations used previously were applied to the primed and resistant uterus they produced a motor response and thus the inhibitory effect had been reversed (Fig. 3). The effect of varying the time of addition was investigated; when the substances were added within 5 min of the onset of isoprenaline inhibition they produced no effect, but a motor effect was observed when the addition was delayed until just before the expected time of escape (7–15 min) from isoprenaline inhibition (Fig. 4).

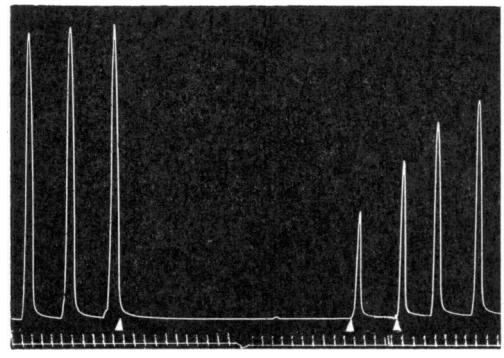

Fig. 3. Oestrous uterus. First arrow: isoprenaline, 100 ng/ml.; second arrow: phenylephrine, 1 μg/ml.; third arrow: wash. Time marks 30 sec.

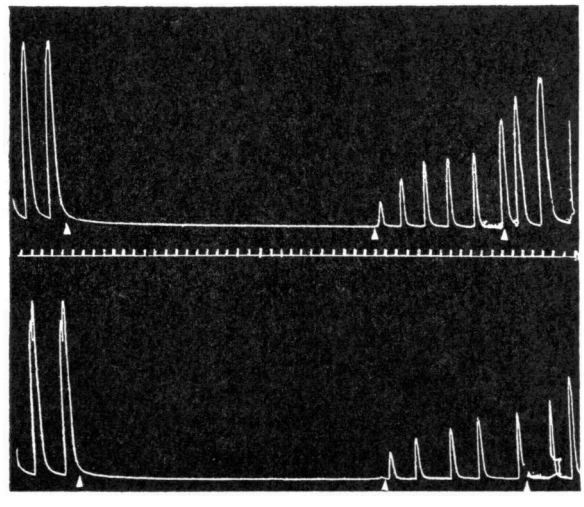

Fig. 4. Both tracings obtained on the same horn of an oestrous uterus. First arrow: in both cases isoprenaline, 100 ng/ml. Second arrow: top tracing noradrenaline, 100 ng/ml.; lower tracing adrenaline, 100 ng/ml. Third arrow: wash. Time marks 30 sec. Note the same magnitude of motor response although the inhibitory action of adrenaline in this concentration is far greater than that of noradrenaline.

Effect of blocking agents on the response of the uterus to motor and inhibitory agents

With the object of elucidating the mechanism of the motor response during isoprenaline resistance the effects of the following blocking agents were studied. Phentolamine and phenoxybenzamine (pure α-blockers, Rudzik & Miller, 1962), dihydroergotamine and hydergine (mixed α- and β-blockers), pronethalol (pure β-blocker), and bromolysergic acid (an inhibitor of 5-hydroxytryptamine receptors). The interaction of these agents with isoprenaline, adrenaline, noradrenaline, phenylephrine and 5-hydroxytryptamine was first examined in the unprimed uterus. Not more than nine drugs were used in any one experiment. The absence of a motor response after the addition of several drugs might be due to cumulative toxicity. As a control oxytocin was added at the end of

the series; the failure of a contraction to occur showed that the uterus was incapable of responding to a motor agent. As a result of the application of this criterion one experiment out of a total of 235 was rejected. The effects of the same agents were then investigated in a uterus which had been primed with isoprenaline. The results are shown in Table 1.

(i) *Unprimed uterus.* Addition of adrenaline, noradrenaline and phenylephrine caused inhibition as stated above.

Phentolamine (10 μg/ml.) caused some increase in the rate of contraction; the motor action of 5-hydroxytryptamine was completely prevented but the actions of the other motor and inhibitory agents were not prevented. The inhibitory action of noradrenaline was potentiated.

Phenoxybenzamine (100 ng/ml.) did not affect the intrinsic rhythm; the motor effect of 5-hydroxytryptamine was prevented and the inhibitory effects of isoprenaline, adrenaline, noradrenaline and phenylephrine were potentiated.

Dihydrogenated ergot alkaloids: dihydroergotamine (100 ng/ml.) and hydergine (200 ng/ml.) inhibited the motor effect of 5-hydroxytryptamine; the inhibitory effects of isoprenaline and phenylephrine were unaffected, and those of adrenaline and noradrenaline were potentiated.

Pronethalol (50–200 ng/ml.) had either a slight or no effect on the intrinsic rhythm; it prevented the actions of those drugs which were inhibitory but not those which were motor.

Bromolysergic acid (100–500 ng/ml.) increased the frequency of contraction. It prevented the motor action of 5-hydroxytryptamine and the inhibitory actions of noradrenaline in three of five and of phenylephrine in two of five instances. The inhibitory actions of adrenaline and isoprenaline were unaffected.

(ii) *Primed uterus.* The effects of adrenaline, noradrenaline and phenylephrine were reversed and they now had a motor effect. The actions of 5-hydroxytryptamine and oxytocin were unchanged.

Phentolamine (10 ng/ml.). The motor effect of phentolamine and its inhibition of the motor effect of 5-hydroxytryptamine were unaffected by priming but the motor actions of adrenaline, noradrenaline and phenylephrine were now inhibited by phentolamine.

Phenoxybenzamine (100 ng/ml.). The effect on the response to 5-hydroxytryptamine was unchanged. The motor effects of adrenaline (Fig. 5), noradrenaline and phenylephrine were inhibited.

Dihydrogenated ergot alkaloids. Hydergine (200 ng/ml.) still inhibited the motor effect of 5-hydroxytryptamine, and the motor actions of adrenaline, noradrenaline and phenylephrine were also inhibited. Dihydroergotamine (100 ng/ml.) still inhibited the motor response to 5-hydroxytryptamine, and the motor responses to adrenaline, noradrenaline and phenylephrine were inhibited in two of five instances for each drug.

Higher concentrations of dihydroergotamine caused too great a motor effect for any blocking action to be assessed.

Pronethalol (50–200 ng/ml.). The effect on the response to 5-hydroxytryptamine was unchanged. None of the motor responses was inhibited.

TABLE 1

THE EFFECT OF BLOCKING AGENTS ON THE RESPONSE OF THE INDUCED OESTROUS UTERUS TO MOTOR AND INHIBITORY AGENTS

+ = Complete blockade. − = No blockade. I = Inhibition of the uterus by the agonist. M = Contraction of the uterus by the agonist. P = Potentiation of the inhibitory effect of the agonist. Each symbol represents one experiment

			Receptors blocked					
			α		α and β		Tryptamine	β
	Agonists	Action on Uterus	Phentolamine (10 μg/ml.)	Phenoxybenzamine (100 ng/ml.)	Dihydroergotamine (100 ng/ml.)	Hydergine (200 ng/ml.)	Bromolysergic acid (100–500 ng/ml.)	Pronethalol (50–500 ng/ml.)
Unprimed uterus	Isoprenaline 100 ng/ml.)	I	−−−−−	−−−−−	−−−−−	−−−−−	−−−−−	+++++
	Adrenaline (100 ng/ml.)	I	−−−−− P	−−−−− P	−−−−− P	−−−−− P	−−−−−	+++++
	Noradrenaline (100 ng/ml.)	I	−−−−− P	−−−−− P	−−−−− P	−−−−− P	−−+++	+++++
	Phenylephrine (1 μg/ml.)	I	−−−−−	−−−−− P	−−−−−	−−−−−	−−−++	+++++
	5-Hydroxytryptamine (100 ng/ml.)	M	+++++	+++++	+++++	+++++	+++++	−−−−−
	Oxytocin (0·002 U./ml.)	M	−−−−−	−−−−−	−−−−−	−−−−−	−−−−−	−−−−−
Primed uterus	Adrenaline (100 ng/ml.)	M	−+++++	−+++++	−−−++	−++++	−++++	−−−−−
	Noradrenaline (100 ng/ml.)	M	+++++	−++++	−−−++	−++++	+++++	−−−−−
	Phenylephrine (1 μg/ml.)	M	−++++	+++++	−−−++	−++++	−++++	−−−−−
	5-Hydroxytryptamine (100 ng/ml.)	M	+++++	+++++	−++++	−++++	+++++	−−−−−
	Oxytocin (0·002 U./ml.)	M	−−−−−	−−−−−	−−−−−	−−−−−	−−−−−	−−−−−

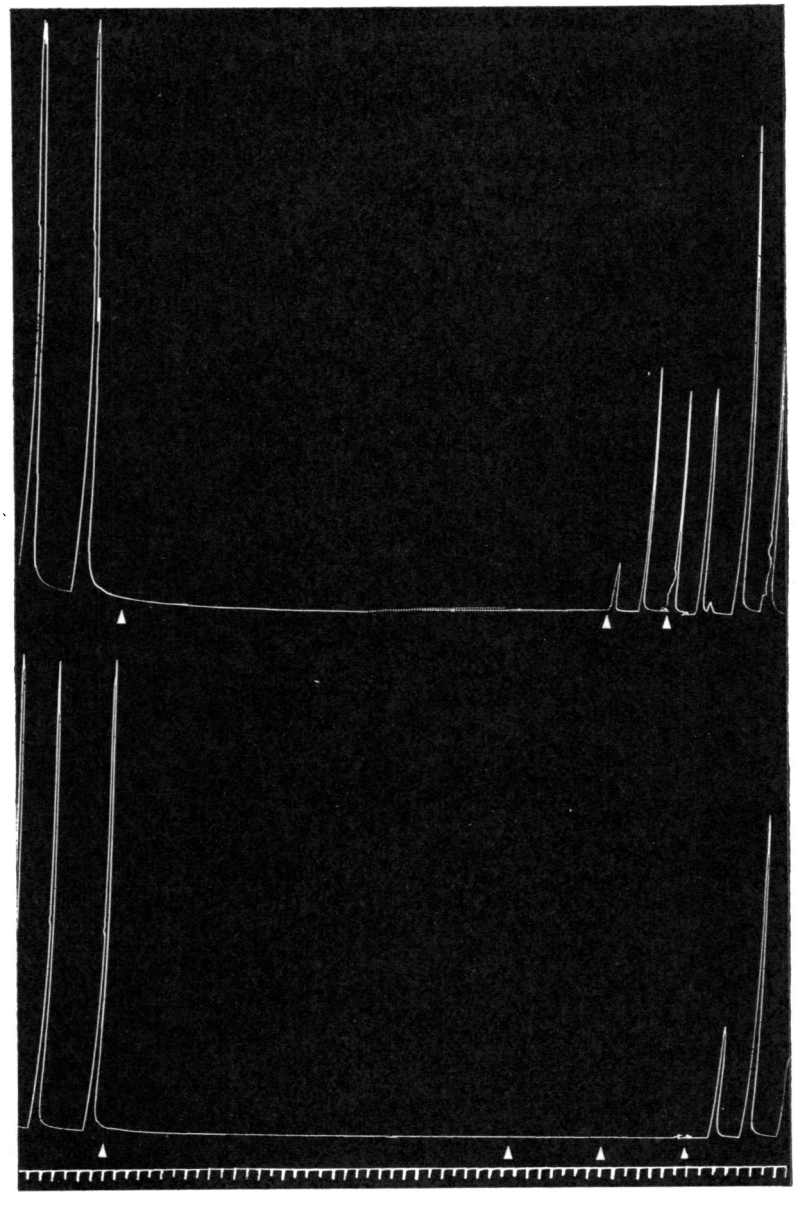

Fig. 5. Both tracings obtained on the same horn of an oestrous uterus. Both tracings: first arrow: isoprenaline, 100 ng/ml.; upper tracing: second arrow: adrenaline, 100 ng/ml.; third arrow: wash. Lower tracing: second arrow: phenoxybenzamine, 100 ng/ml.; third arrow: adrenaline, 100 ng/ml.; fourth arrow: wash. Time marks 30 sec.

Bromolysergic acid (100 ng/ml.) had a motor effect in nine of 25 experiments (36%). The effect on the responses to 5-hydroxytryptamine was unchanged. The motor responses to adrenaline, noradrenaline and phenylephrine were prevented (Fig. 6).

Isoprenaline resistance and the reversal phenomenon in different functional states of the uterus

On the basis of the results obtained with uteri from animals in induced oestrus (group 1), a comparative survey was made of isoprenaline resistance and the adrenaline reversal in uteri from animals in other functional states (groups 2, 3 and 4). After the addition of isoprenaline the preparations were observed for a maximum period of 1 hr ; if inhibition persisted for this length of time and a motor response to adrenaline could not be obtained at this time it was assumed that escape would not occur.

Escape from isoprenaline occurred in 17 of 23 uteri in induced oestrus, one of 16 in natural oestrus, three of 20 in the progestational phase and 15 of 18 from pregnant animals. There was no statistical difference between the incidence of escape in the groups of induced oestrus and pregnant animals, or between animals in natural oestrus and the progestational phase. However, the incidence was significantly ($P<0.001$) lower in natural oestrus and the progestational phase in comparison with induced oestrus and pregnancy.

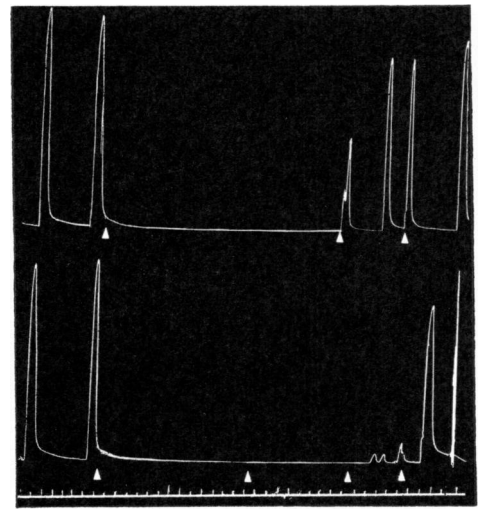

Fig. 6. Both tracings obtained on the same horn of an oestrous uterus. Both tracings: first arrow: isoprenaline, 100 ng/ml. Upper tracing: second arrow: adrenaline, 100 ng/ml.; third arrow: wash. Lower tracing: second arrow: BOL, 100 ng/ml.; third arrow: adrenaline, 100 ng/ml.; fourth arrow: wash. Time marks 30 sec.

DISCUSSION

Detailed consideration of the results presented in Table 1 leads to certain conclusions. As inhibition by adrenaline, isoprenaline, noradrenaline and phenylephrine was abolished by the β-blocking agent pronethalol, but was unaffected or potentiated by the α-blocking

agents, α-inhibitory receptors, as postulated by Rudzik & Miller (1962), are not present in the rat uterus. This conclusion is in agreement with the work of Levy & Tozzi (1963) and Diamond & Brody (1966).

In the primed uterus, however, these agents become motor, and their effects were usually prevented by the α-blockers but not by the β-blocker. This could be explained according to the accepted receptor theory by postulating that isoprenaline occupies the β-receptors, thus allowing the effects upon the α-receptors to become apparent. Addition of agents with more definite actions on these receptors, such as adrenaline and noradrenaline, increased the height and frequency of the contractions during escape. Similar results were obtained with the mixed α- and β-blocking agents in the primed and unprimed uterus, with the exception of dihydroergotamine, which in the primed uterus was not effective in all cases.

The action of 5-hydroxytryptamine was motor in both the unprimed and the primed uterus. The height of the contraction was not significantly reduced in the presence of the inhibitory action of isoprenaline, so it can be assumed that the action of 5-hydroxytryptamine remained unchanged by priming. It was prevented by the 5-hydroxytryptamine blocking agent (BOL) and by the mixed α- and β-blocking agents in both the primed and the unprimed uterus. A possible explanation of this would be that the α- and the mixed α- and β-blocking agents used in the present work are not specific in their α-blocking actions but also block tryptamine receptors. In the unprimed uterus the inhibitory action of noradrenaline and phenylephrine was blocked by the 5-hydroxytryptamine blocking agent in five of 10 experiments but the inhibitory action of adrenaline and isoprenaline remained unchanged. In the rat uterus the inhibitory action of both noradrenaline and phenylephrine is weak, and it may be that they are readily displaced from the β-receptors by a number of blocking agents acting on related receptors. In the primed uterus the motor action of adrenaline, noradrenaline and phenylephrine was blocked by the 5-hydroxytryptamine blocking agent. This finding, in addition to the fact that the action of 5-hydroxytryptamine was blocked by the α- and the mixed α- and β-blocking agents, but not by pronethalol in the primed and the unprimed uterus, suggests that 5-hydroxytryptamine may somehow be concerned in the α-motor pathway. It is therefore postulated that uteri from rats treated with oestrogens or in late pregnancy, during isoprenaline resistance, contain a motor receptor for both 5-hydroxytryptamine and adrenaline which is either not detectable or absent in uteri from rats in natural oestrus or treated with progesterone. This receptor appears to be occupied by both α-blockers and 5-hydroxytryptamine blockers but not by β-blockers. As it is impossible to describe this receptor as either α or tryptamine from the existing evidence, it is proposed that, until further work elucidates the exact nature of this receptor, it should be called the E-receptor.

The discrepancies in the literature which are summarized in Table 2 may be due to insufficient control of the functional state of the uterus. A motor response to adrenaline during β-blockade with pronethalol was obtained by Diamond & Brody (1966), who treated rats with oestrogen 48 hr before use, but not by Levy & Tozzi (1963), who only treated for 24 hr. In the present work a period of 40–45 hr was selected, and the results were in agreement with Diamond & Brody (1966). On the other hand Levy & Tozzi (1963) were unable to detect an excitatory receptor in the non-pregnant untreated uterus

TABLE 2

ANALYSIS OF RECEPTORS REPORTED IN THE RAT UTERUS IN ACCORDANCE WITH FUNCTIONAL STATE

Authors	Preliminary treatment	Functional state of uterus	Postulated receptors
Ahlquist (1948)	Not stated	Not stated	Inhibitory (β) Excitatory (α)
Rudzik & Miller (1962)	Diethylstilboestrol 15 mg/150 g rat, time given not stated	Oestrogen primed	Inhibitory (β) Inhibitory (α)
Levy & Tozzi (1963)	1. Diethylstilboestrol 10 μg/100 g rat, 24 hr before experiment 2. None 3. None 4. None	1. Oestrogen primed 2. Dioestrus 3. Prooestrus 4. Natural oestrus	Inhibitory (β)
Diamond & Brody (1966)	Oestradiol benzoate 50 μg/200 g rat, 48 hr before experiment	Oestrogen primed	Inhibitory (β) Excitatory (α)
Present work	1. Diethylstilboestrol 500 μg/220 g rat, 40–45 hr before experiment 2. Ovariectomized and progesterone 3. None 4. None	1. Induced oestrus 2. Progestational 3. Natural oestrus 4. Pregnant	Inhibitory (β) Excitatory (E) Inhibitory (β) Inhibitory (β) Inhibitory (β) Excitatory (E)

in three of the stages of the oestrous cycle. This would be comparable to the present findings for uteri in natural oestrus and in the progestational state. If the present work is interpreted according to the receptor theory it would be necessary to postulate that the E-receptor in the rat uterus appears and disappears according to the functional state of the uterus, whereas the inhibitory receptor remains unchanged. If, however, receptors are closely related to enzymes, as suggested by Sutherland & Rall (1960), treatment with stilboestrol for a period of 40 hr would be adequate to permit an enzyme to be induced, in other words for the E-receptor to appear in the uterus in induced oestrus. The blood oestrogen levels rise steeply throughout pregnancy in women (Roy & Mackay, 1962), and if this also occurs in rats, the high levels of oestrogen might be able to induce the enzyme which is postulated to be the E-receptor. Final evaluation of this hypothesis will have to await the demonstration of an enzyme which varies in activity in the postulated manner in the different functional states of the uterus.

SUMMARY

1. Treatment with isoprenaline *in vitro* (priming) and blocking agents was used as a means of investigating receptor mechanisms in the rat uterus in different functional states.

2. The inhibitory response to adrenaline, noradrenaline and phenylephrine became motor after priming. A motor response to adrenaline was easily elicited in stilboestrol-treated uteri and uteri from animals in late pregnancy but not from animals in natural oestrus or ovariectomized and treated with progesterone.

3. The motor responses to adrenaline, noradrenaline and phenylephrine in the presence of isoprenaline were blocked by an α-, a mixed α and β-, and a 5-hydroxytryptamine-blocking agent. The latter was used in a concentration which blocked 5-hydroxytryptamine in the unprimed and primed uterus. The α- and mixed α- and β-blocking agents blocked 5-hydroxytryptamine in the unprimed and the primed uterus.

4. On the basis of the receptor theory it is concluded that there is an excitatory receptor in the rat uterus which is present only in uteri from stilboestrol-treated and pregnant animals. It is concluded that 5-hydroxytryptamine may be concerned in the α-motor pathway. The nature of the excitatory receptor cannot be definitely identified; pending further clarification it is proposed that it should be called the E-receptor.

5. It is postulated that treatment with stilboestrol for 40 hr or the high levels of oestrogens which occur in the latter half of pregnancy may induce an enzyme which may be the E-receptor.

6. The conflicting statements in the literature concerning the nature of the catecholamine receptors in the rat uterus appear to be due to insufficient control of the functional state of the uterus.

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