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EFFECTS OF ADRENALINE ON THE ISOLATED UTERUS OF THE CAT

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Over fifty years ago Dale (1906) showed that the response of an isolated strip of cat uterus to added adrenaline varies with the state of the animal in relation to pregnancy. Uteri from virgin cats or non-pregnant cats in oestrus or in anoestrus are inhibited by adrenaline. If there is spontaneous activity it ceases; if there is high tone it relaxes; if there is no movement and the muscle is relaxed the quiescent condition is not disturbed and may be prolonged by adrenaline. If the virgin cat is injected subcutaneously with a preparation of oestrogenic hormone the uterus enlarges, becomes congested and when isolated is frequently found to be active, but the response to added adrenaline is unaltered. If the cat from which it is taken is pregnant, as Dale showed, the uterine muscle responds to adrenaline with contraction. This change in the nature of the response develops shortly after pregnancy begins and progresses with the gain in progesterone control. It is lost again with involution of the uterus post partum. Similarly, the altered response to adrenaline may be induced by injecting an oestrous cat with progesterone and it develops with proliferation and other evidences of pregnancy hormonal action.

We have explored the possibility of altering the response to adrenaline of an isolated strip of cut uterus by preparations from tissues of the same or other female cats.

METHODS

Female cats were collected in pairs as follows: (*a*) one pregnant one non-pregnant, both parous, between February and September; (*b*) neither pregnant, both parous, at any time of the year: these cats were given 0·5 mg stilboestrol daily for 10 days by subcutaneous injection; then one was injected with stilboestrol, the other with progesterone 2 mg/day for a further 10 days. (*c*) One pregnant, one virgin, 4–6 months of age, reared in captivity. In some cases the virgin cat was injected for 10 days with stilboestrol as above. (*d*) One virgin, reared in captivity for 6 months, one pseudo-pregnant, parous, injected with stilboestrol and then progesterone as in (*b*).

The cat was lightly anaesthetized with ethyl chloride spray and exsanguinated, and

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the uterus (without ovaries) removed. A strip of the uterus was mounted in Locke's solution at 37° C., bubbled with 95 % O_2 + 5 % CO_2, and its tone and movement were recorded with a frontal-writing lever. The size of bath was usually 10 ml., but 30 ml. for two specimens together. The response to adrenaline 10^{-6}–10^{-8} was recorded under certain sets of conditions detailed below. Extracts were made initially by grinding tissue with washed powdered glass, latterly in homogenizers, in 2 ml. Locke's solution/g wet tissue. Skeletal muscle, lung, liver and uterus were prepared in this way and added in varying amounts to the bath for 10–15 min before testing with adrenaline. Some extracts were boiled and cooled and the filtrate used. Progesterone 2 mg in oil was emulsified with warm Locke's solution 10 ml. and concentrate of Tween 80 0·3 ml. and put into the bath containing oestrous uterus for 1 hr before testing with adrenaline. The combinations of isolated uteri and extracts examined were as follows: (*a*) virgin; naturally oestrous parous non-pregnant; oestrogen-injected virgin; oestrogen-injected anoestrous parous—alone; (*b*) the same, with, in the bath, a 1–2 g piece of skeletal muscle, liver, gut or lung; (*c*) the same with, in the same 30 ml. bath, a strip of pregnant or pseudopregnant uterus as in (*e*). The tissues were suspended from separate levers which had no contact, and remained for 2–3 hr together before testing with adrenaline; (*d*) the same, with, in the 10 ml. bath, homogenate of skeletal muscle, liver, lung or of uterus equivalent to 1 g of tissue. The homogenized uterus was either (1) oestrous non-pregnant (natural or injected with stilboestrol); (2) pregnant; (3) pseudopregnant adult (injected with stilboestrol and then progesterone). The extract was added and a variable time allowed to elapse before adding adrenaline. The bath might contain atropine sulphate 10^{-8} or mepyramine maleate 10^{-8}. To one piece of an oestrous uterus, the extract of progesterone-proliferated uterus was added as described, while to another piece of the same oestrous uterus ergotamine tartrate 10^{-5} or phenoxybenzamine 10^{-6} was first administered for 15 min; (*e*) a strip of pregnant uterus cleaned of its endometrium, or of pseudopregnant uterus (progesterone-injected cats)—alone.

RESULTS

When the isolated uterus of a parous cat taken in the spring season (natural oestrous) was treated with adrenaline, spontaneous rhythm ceased and tone, if present, was relaxed. The sensitivity of the tissue was not great and a concentration of 10^{-7} was often needed. A similar response was obtained with the uterus of oestrogen-injected cat which showed much spontaneous activity. Virgin cat uterus often had no spontaneous rhythm and little tone, in which case it remained apparently unaffected by added adrenaline. The patterns of spontaneous activity varied widely, but in no case did adrenaline 10^{-6} fail to produce or maintain a prolonged relaxation and quiescence. The presence in the bath of a piece of liver, lung, gut or skeletal muscle did not modify the response of the non-pregnant uterus to adrenaline.

If the uterus was taken from a pregnant cat, especially if late in term, and the contents and lining were removed, addition of adrenaline 10^{-8} caused spontaneous rhythm to speed up or start; tone developed or increased. The pseudo-pregnant uterus reacted similarly and both these tissues were more sensitive to adrenaline than is non-pregnant uterus; 10^{-8} almost always produced a clear response and 10^{-6} invariably produced spasm.

If a larger strip of pregnant cat uterus was mounted in the same bath with a smaller strip of non-pregnant uterus from a parous cat, and left for 2–3 hr, various changes in tone and spontaneous rhythm of one or other specimen occurred. Very often both became quiescent. If adrenaline was then added the pregnant uterus gave a motor response but the non-pregnant uterus also made a motor response—a change or reversal of the response expected. This change has been reported and illustrated by Graham & Gurd (1959). Washing restored the customary inhibitor response to the non-pregnant uterus. Similarly, if the larger tissue was from a pseudo-pregnant cat (progesterone-treated) and the smaller strip from an oestrogen-injected cat, they both responded to added adrenaline with a contraction. If the oestrous portion was showing spontaneous activity this increased in rate and diminished in extent; if it was fully relaxed it showed a rise in tone and movement began. These reactions, taken from different specimens on separate occasions, are shown in Fig. 1. Only pregnant or pseudo-pregnant uterus will bring about the complete reversal of the response to adrenaline in the uterus of an oestrogen-injected cat. Other tissues or non-pregnant oestrous or anoestrous uterus have no effect, nor does the addition to the bath of progesterone in oil. If the non-pregnant segment was from an immature virgin cat the pattern of spontaneous activity altered but there was no 'reversal' of the action of adrenaline.

If a piece of uterus from a pregnant cat or from a cat injected as described with progesterone is macerated or homogenized with cold saline and centrifuged or filtered and the supernatant or filtrate taken, this may be termed a simple extract. When a portion of simple extract was added to a bath containing a piece of non-pregnant parous uterus and adrenaline, in an amount which previously elicited an inhibitor response added after some 10–15 min, the response was an excitor one. Extracts of most tissues have an oxytocic effect on isolated non-pregnant parous cat uterus (Collip, 1922). If the extracts were made from liver, lung, gut, muscle, or non-pregnant uterus, the reaction to adrenaline of the isolated uterus to which the extracts were added might be antagonized during the oxytocic phase but not reversed (see Fig. 2, Nos. 3 and 4). Simple extracts of pregnant or progesterone-proliferated uterus are also oxytocic but in addition they 'reverse' the response to adrenaline. The reversal can be demonstrated while the oxytocic effect is in progress, after it has passed off (Fig. 2, No. 1) or before it has developed (Fig. 2, No. 2) as there is usually a latency. If the oestrous uterus was in rhythmic movement this was speeded up, or increased in excursion; if it was quiescent it contracted and rhythmic activity followed.

A variable minimal amount of extract was needed before reversal occurred and increasing doses had to be added in order to reverse any

given piece of non-pregnant tissue. Not all extracts were effective in doses such that the oxytocic effect did not cause prolonged spasm and obscure the result. The oxytocic effect was unaffected by boiling the extract, but was reduced if mepyramine maleate or atropine sulphate 10^{-8} was first added to the bath. Reversal was not affected. Prolonged soaking of

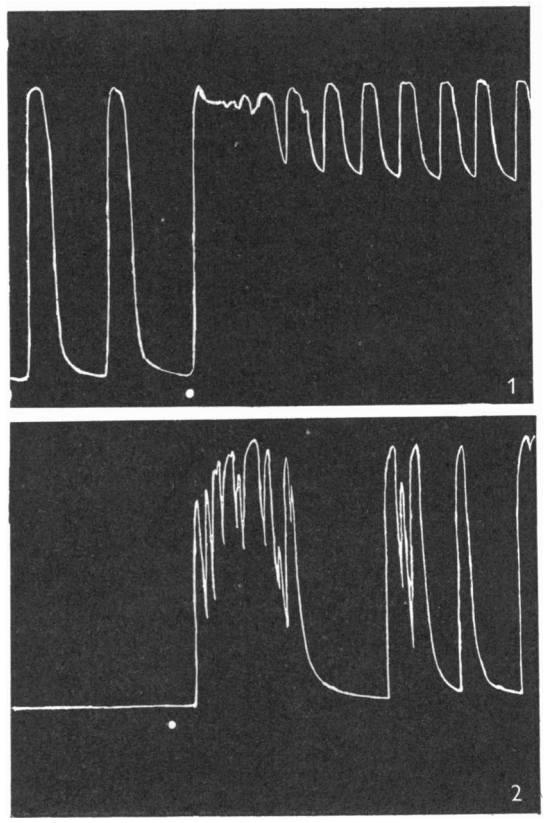

Fig. 1. Isolated strips of uterus from two cats in oestrus. With each, in the same bath, there has been a large portion of uterus from a cat injected with progesterone. The response to added adrenaline of the non-pregnant uterus is now reversed. (1) Spontaneous contractions speeded and tone increased; (2) quiescent uterus roused to activity.

the oestrous uterus in extract rendered it insensitive to stimulants. This is a phenomenon unrelated to 'reversal', but may obscure results. The 'reversal' effect, once demonstrated, was removed by washing, but some accumulation must occur, as lesser amounts of the same extract were effective in consecutive trials on the same tissue. Prior addition of ergotamine tartrate or of phenoxybenzamine to oestrous tissue prevented the development of reversal to adrenaline by extract of progesterone-pro-

liferated uterus. If the non-pregnant uterus was from a virgin cat, extract of pregnant uterus antagonized the response to adrenaline but did not reverse it. Extract of progesterone-proliferated uterus also had an oxytocic effect, but brought about a true reversal to adrenaline if the virgin

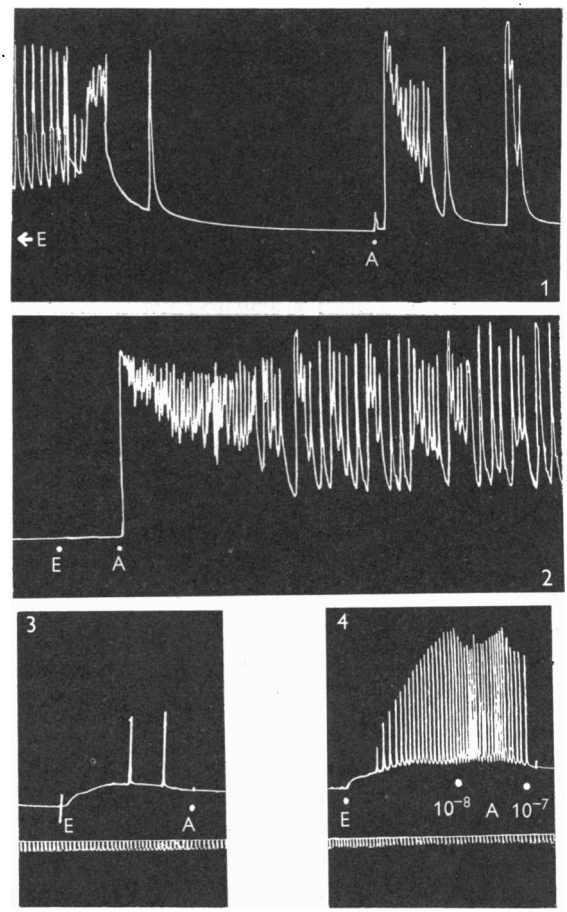

Fig. 2. Isolated strips of uterus from cats in oestrus. The effects of adding extracts of tissue and then adrenaline. (1) and (2) extract of 1 g uterus of cat treated with progesterone; action of adrenaline reversed (1) after and (2) before onset of oxytocic effect of extract. (3) Extract of 1 g skeletal muscle. (4) Extract of 1 g non-pregnant uterus; action of adrenaline not reversed. Time marker, 30 sec.

cat was well developed (6 months of age; 3 experiments) but not if it was very immature (3 experiments) and inactive when isolated. The presence of a large piece of non-pregnant cat uterus in a bath with a small piece of pregnant cat uterus has no effect on the response of the latter to added adrenaline.

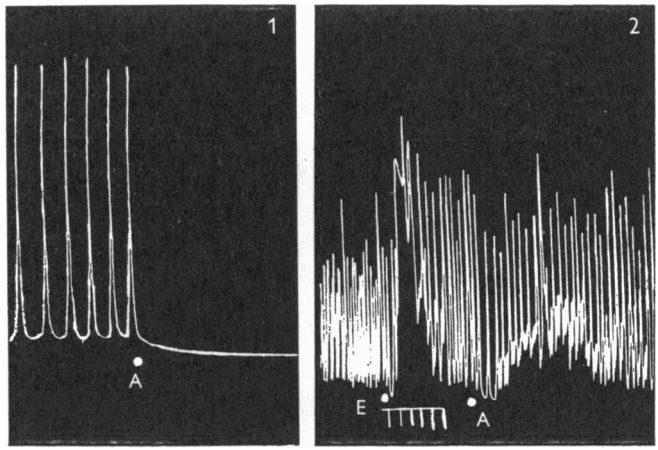

Fig. 3. Isolated strip of uterus from virgin cat (6 months old). (1) Adrenaline inhibits activity. (2) After the oxytocic effect of extract of 1 g uterus from a cat treated with progesterone has passed off adrenaline had a motor action. Time marker, 1 min.

DISCUSSION

McSwiney & Brown (1926) studied the response of several tissues, including rabbit stomach and uterus, to added adrenaline in various conditions of tone of the preparation. They concluded that 'the mechanism controlling the augmentor and inhibitor response to adrenaline may be present in all preparations of smooth muscle capable of active contraction and relaxation'. As far back as 1919 Cow examined non-pregnant uterus from guinea-pig. Normally he found this muscle inhibited by added adrenaline but after a soak in pituitrin (an extract of whole gland which caused a spasm) the muscle contracted to added adrenaline. Collip's work (1922) is also important. He found that a watery extract of most tissues was oxytocic. 'Extracts, in addition to causing intense stimulation of the uterus antagonized the inhibitory action of adrenaline on such uteri as are normally inhibited by this latter substance, as in the case of the rat and guinea-pig (both virgin and gravid) and the virgin rabbit and dog'. He does not mention or illustrate a true reversal of the response to adrenaline but speaks of 'an instance of certain tissue constituents acting selectively upon the inhibitory nervous apparatus of a tissue irrespective of whether it is of the sympathetic or parasympathetic type'.

The present experiments are concerned with contraction in isolated uterus rather than with changes in tone. The results imply that something is present in pregnant or progesterone-proliferated cat uterus which can be extracted by water and which can diffuse in a bath. This substance,

which may be a water-soluble, quick-acting form of pregnancy hormone, does not behave like progesterone. It is capable of penetrating oestrous uterine muscle and altering it so that the response to adrenaline becomes a motor one. Immature muscle which may not have experienced the influence of oestrogen is not affected. The site of action is such that anti-adrenaline compounds (ergot alkaloid or phenoxybenzamine) prevent it.

SUMMARY

If non-pregnant cat uterus is treated with an extract of pregnant cat uterus the action of adrenaline on it is reversed. This effect is prevented by phenoxybenzamine.

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