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TRANSMITTER RELEASED IN THE CAT UTERUS BY STIMULATION OF THE HYPOGASTRIC NERVES

BY MARTHE VOGT

From the Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge

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The object of the present work was twofold. First, to find out whether there are occasions when adrenaline, and not noradrenaline, may act as a transmitter at mammalian adrenergic nerve endings. Secondly, to see whether there was a change in the nature of the transmitter at the time when the cat's uterine response to hypogastic nerve stimulation turned from relaxation in the non-pregnant to contraction in the pregnant state.

METHODS

Female cats were used; some were virgin, others had had litters or were pregnant. They were anaesthetized with ether followed by intravenous chloralose. A small quantity of blood (5 ml./kg. body weight) was usually taken from the left carotid artery, and kept (after addition of heparin) for reinfusion during collection of uterine blood. Since such blood would not be free of catecholamines, care was taken to infuse the same volume of blood during each collection period.

The cat was eviscerated, both adrenal glands were extirpated and the vessels of one (usually the left) kidney were tied as near the kidney as possible. In most cats the ovarian vein enters the renal vein sufficiently far from the hilum to permit cannulation of the lateral part of the renal vein and application of a small bulldog clamp without obstruction to the blood flow from the ovarian vein. However, a valve not infrequently prevents 'retrograde' collection of uterine blood; in such instances the cannula was inserted directly into the ovarian vein and collection was continuous throughout the experiment, the blood being returned to the cat when intervals between collection periods were necessary. In some experiments the tributaries coming from the ovary were ligated in order to avoid undue dilution of the uterine blood. The uterine horn from which the blood was being collected was attached to a lever to record movement in much the same way as described by Dale (1906); the hypogastric nerve of the same side was cut centrally, threaded through a bipolar fluid electrode and stimulated with square pulses of 1 msec duration at a frequency varying from 10 to 30/sec. Voltage was supramaximal. When the dissection was complete, heparin (1000 i.u./kg) was injected into the cannulated jugular vein and the carotid blood pressure was recorded by manometer. The abdomen was filled with warm saline solution and kept warm with an infra-red lamp. Uterine responses were poor unless normal body temperature was maintained in the abdominal cavity.

The blood was collected into ice-cooled tubes and centrifuged as soon as possible at 3000 rev/min; the plasma was extracted and adrenaline and noradrenaline were separated on paper as described previously (Vogt, 1952). The eluted amines were assayed by the blood-pressure rise elicited in the pronethalol-treated pithed rat (Vanov & Vogt, 1963). When drugs

had been injected into the cat, it was necessary to ascertain whether they could interfere with the assay. Cocaine, pronethalol, dichloroisoproterenol and desmethylimipramine, the drugs used in these experiments, travel near the front of the phenol/HCl chromatogram, the R_F value being 0·85–0·9. They are therefore localized far ahead of adrenaline, which is the faster travelling of the two catecholamines, and cannot contaminate the eluates used for assay. In the early experiments, in which an attempt had been made at shortening the extraction procedure, there was often some depressor material in the adrenaline fractions which 'masked' the effect of small doses of adrenaline, so that the threshold of the method was higher than in the later experiments, and low concentrations of adrenaline may have been missed. On the other hand, when interfering substances were absent and the rat was of more than average sensitivity, responding, for example, to 0·125 ng adrenaline with a rise in blood pressure distinguishable from that produced by an injection of saline, it was possible to determine as little as 0·4 ng per sample.

Catecholamine concentrations were not corrected for recoveries (which ranged from 50 to 70 %) and have been expressed in terms of the base.

The following drugs were used, all as hydrochlorides: Cocaine, pronethalol (2-isopropylamino-1-(2-naphthyl) ethanol), desmethylimipramine ($N(\gamma$-methylaminopropyl) iminodibenzyl), and dichloroisoprenaline (dichloroisopropylnoradrenaline). The doses are expressed as weights of the salts.

RESULTS

Amines in control samples

It was usually possible to detect noradrenaline, and sometimes adrenaline, in control samples of uterine blood taken from non-pregnant cats no less than 1 hr after extirpation of the adrenals. This was at first thought to indicate constant leakage from the uterine tissue, but estimation of the amine content of arterial blood at the end of the last five experiments showed that there was approximately the same concentration in arterial blood and uterine effluent, so that the catecholamines found there represented, in fact, circulating amines. The values ranged from 0·5 to 2 ng noradrenaline per ml. arterial plasma; the highest adrenaline content was 1 ng/ml.

With two exceptions the adrenaline content of control samples of uterine blood was lower than that of noradrenaline. In all cats uterine responses to intravenous injection of adrenaline and noradrenaline were recorded, and the approximate ratio of doses producing the same motor effect was determined. These tests were usually carried out after the collection of blood from the ovarian vein had been completed. If they were made at the beginning of the experiment, an interval of about 0·5 hr was allowed between the last injection and the taking of the first sample. Even so, contamination of the uterine blood with noradrenaline occasionally occurred, because the non-pregnant uterus is so insensitive to noradrenaline that large doses (20–40 μg) had to be administered in order to produce an effect.

Release of amines

During stimulation of the hypogastric nerve, catecholamines increased in the uterine blood in seventeen experiments, whereas no change occurred in five. An interesting point arises from an analysis of the five failures: two were cats in advanced pregnancy, and three had recently littered; it is likely that uptake by the tissues of amines released as a result of stimulation was increased, and escape into the circulation reduced, when the uterine tissue mass was large or very vascular. Some samples were taken immediately after the stimulation period, but the effect of stimulation had usually worn off.

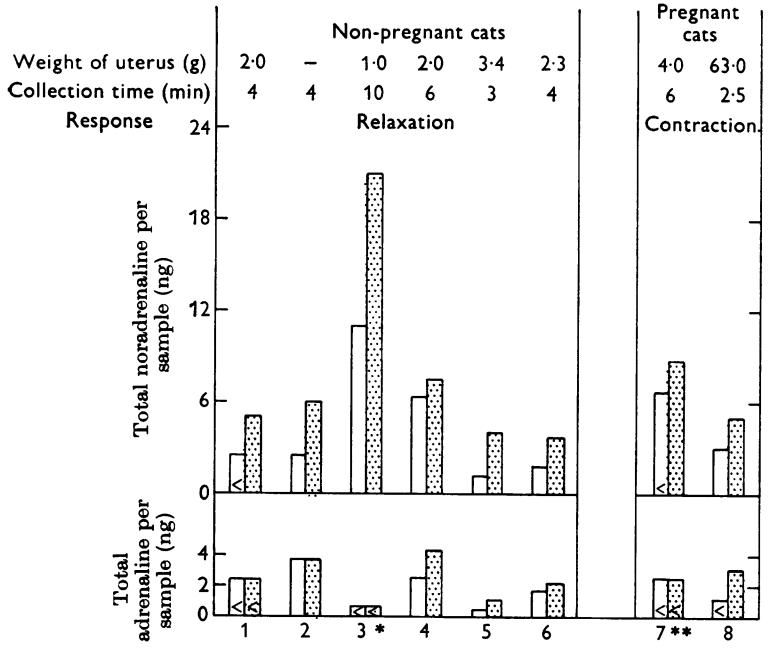

Fig. 1. Adrenaline and noradrenaline content of blood from one ovarian vein of adrenalectomized cats. Open columns before, stippled columns during electrical stimulation of one hypogastric nerve. The columns represent the total uncorrected catecholamines (in ng) determined during periods indicated as 'collection time'. The < sign at the bottom of a column designates that less than the indicated quantity of amines was present. * Stimulation of the hypogastric nerve produced severe vasoconstriction but little relaxation of uterus. ** Resorbed pregnancy.

Experiments without the use of drugs which inhibit catecholamine 'uptake'. In eleven cats only noradrenaline was found to have been released by nerve stimulation, and in six cats both amines were detected. It is quite likely that, particularly in the early experiments (see Methods), adrenaline

was sometimes missed because of the limitations of the technique. In some of the experiments it was possible to assess the importance of the individual catecholamine in the muscular response observed. Thus in Expts. 1, 2, 3, 9*a*, 10*a* and 11*a* (Figs. 1, 2) on non-pregnant cats, and in Expt. 7 on a pregnant cat, the noradrenaline release was large whereas adrenaline was either not released (Expt. 2) or, at most, might have constituted a very small fraction of the total amines. Though injected adrenaline was between 6 and 75 times more potent on the uterine muscle than noradrenaline in these experiments (including the one on a pregnant cat), it appears justified to conclude that the noradrenaline must have had a large share in, if not been entirely responsible for, the response. One might even suppose that the poor relaxation obtained on nerve stimulation in Expt. 3 was due to the fact that adrenaline was probably not released in this animal. The position is reversed in Expts. 4, 5, and probably 6, on non-pregnant cats, as well as in 8 on a pregnant cat. In Expt. 4 more adrenaline than noradrenaline was liberated; in Expts. 5 and 6 the two amines were found in a proportion of about 1 to 4; the ratio of their potencies was > 10 in all these experiments. In the pregnant cat the amounts of the two amines released were about equal, and so was their activity on the muscle. It follows that in these experiments the share of adrenaline in the relaxation of the uterine muscle during nerve stimulation must have ranged from 50 to 95%.

Frequency of stimulation. In their work on release of sympathetic transmitter from the spleen Brown & Gillespie (1957) showed that the output per stimulus was low at 10/sec, reached a maximum at about 30/sec, and fell again when stimulation frequency was further increased. These figures, however, were valid only for short periods of stimulation, 20 sec at a frequency of 10/sec, 7 sec at a frequency of 30/sec. Since the amount of transmitter escaping from the uterus was extremely small, long collection periods of 2·5–10 min were used in the present study, undoubtedly introducing complicating factors such as fatigue of transmission and adverse effects of prolonged and severe vasoconstriction. Relaxation of the muscle was, however, always well maintained during, and often beyond, the period of stimulation, whereas contraction was not maintained. An increase of the frequency of stimulation from that of 10/sec initially used to 20 or 30/sec and a decrease to 6 or 8/sec was tried. Sympathomimetic amines were released at all frequencies, but variation between experiments carried out at the same frequency was so great that no evidence on the effect of altering the frequency was obtained.

Mechanical responses of uterine muscle. As shown in 1910 by Barger & Dale, adrenaline is a much stronger relaxant of the uterus of the non-pregnant cat than is noradrenaline; in the present work the activity ratios

were found to lie between 15 and 75. In the pregnant uterus, on the other hand, the ratio of doses producing the same contraction was about 1, as recently also observed by E. Bülbring (personal communication). Mixed responses and ratios of 4–6·5 were seen in cats which had recently littered or in which the foetuses had been resorbed. In one such cat a low dose of adrenaline and a weak stimulus to the hypogastric nerve relaxed, whilst a larger dose and a stronger stimulus contracted the uterus; in another,

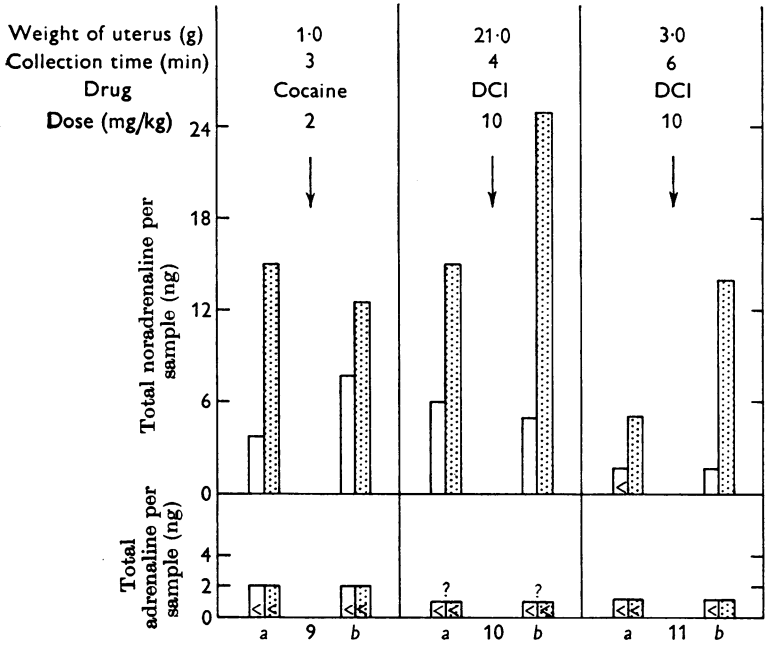

Fig. 2. Effect of cocaine and dichloroisoprenaline (DCI) on catacholamines escaping into the uterine effluent: (*a*) before, (*b*) after the drug. The drugs were injected at the arrows, and the second samples were taken approximately 0·5 hr later. Columns have the same meaning as in Fig. 1. Before the drug was given, there was relaxation of the uterus in response to stimulation of the hypogastric nerve. Following dichloroisoprenaline, there was contraction. The lower limits marked with a query are uncertain because of the presence, in the eluates, of material interfering with the assay.

the uterus relaxed on injection of catecholamines and contracted on hypogastric nerve stimulation, but showed the usual blanching, thus indicating that the hypogastric nerve was functional. Perhaps a weaker stimulus would have produced relaxation, as was seen in the previous post-partum cat. Unfortunately, probably as a result of the presence of a large mass of uterine tissue, no catecholamines were recovered in the uterine blood of the cat in which nerve stimulation and injection of adrenaline had produced opposite effects.

The use of drugs which inhibit catecholamine 'uptake'. An attempt was made to increase the unsatisfactorily small release of amines into the uterine effluent by giving drugs which have been shown to interfere with the uptake of catecholamines by tissue. Increased release of noradrenaline on stimulation of the sympathetic supply to perfused organs has been reported with two of the drugs used, cocaine and dichloroisoprenaline (Huković & Muscholl, 1962; Thoenen, Huerliman & Haefely, 1964); frequency of stimulation had to be low for the effect to occur. The other compounds chosen in the present work were pronethalol, shown by Lindmar & Muscholl (1964) to inhibit uptake of noradrenaline in the rabbit heart, and desmethylimipramine, found by Iversen (1964) to be the most potent inhibitor of uptake in the rat heart. All four drugs affected uterine responses: cocaine and desmethylimipramine caused greater relaxation in response to nerve stimulation; dichloroisoprenaline and pronethalol reversed or abolished relaxations produced by injection of catecholamines or by stimulation of the hypogastric nerve; vasoconstriction was not diminished.

The results with these drugs on the release of transmitter were disappointing. With dichloroisoprenaline (10 mg/kg) the release of noradrenaline was, indeed, doubled in two cats and that of adrenaline raised above threshold in one, but after pronethalol (2 mg/kg) noradrenaline release was small in two and absent in the third cat. Cocaine (1·5 and 2·0 mg/kg, 3 experiments) reduced the release, and desmethylimipramine (3–10 mg/kg, given very slowly, and allowing at least 0·5 hr for the heart to recover from the effect of the drug) enhanced release only in one of four trials. The experiments were therefore discontinued.

DISCUSSION

The experiments reported here suggest that, in the cat's uterus, adrenaline and noradrenaline can function as transmitters. Methodological difficulties made it impossible to state whether adrenaline always shares in the transmission process when the hypogastric nerves are stimulated. The relative amounts of the two amines appearing in the venous blood were subject to large variations, but when the much greater potency of adrenaline as a relaxing substance was taken into consideration it was evident that in some experiments adrenaline must have been the main contributor to the effect produced.

The few successful experiments on pregnant uteri suggest that, as in the non-pregnant state, amines escaping into the effluent sometimes consisted predominantly of noradrenaline, and sometimes to over 50% of adrenaline. Thus any changes taking place during pregnancy in the responses to hypogastric nerve stimulation appear not to involve changes

in the nature of the transmitter. Graham & Gurd (1960) have suggested that a substance is formed in the progesterone-dominated cat uterus which reverses the action of adrenaline.

Experiments of the kind described in this paper do not measure transmitter released, but only 'overflow', i.e. the difference between amount released and amount re-entering the stores. The estimations, therefore, do not give information on the amount of transmitter required per impulse, but for the interpretation of the results the assumption has been made that the two amines are escaping into the effluent in the proportion in which they are released.

A surprising aspect of this work has been the extraordinarily small quantities of transmitter escaping into the general circulation. This indicates a fundamental difference from tissues such as the spleen, which, as had been known since the early work of Peart (1949), releases enough sympathin into the circulation, in response to stimulation of the splenic nerves, for easy detection and estimation. In the uterus the highest amount liberated per min into the circulation was found to be noradrenaline 5 ng and adrenaline 0·6 ng. Either the mechanism for re-uptake of released catecholamines is extremely efficient in the cat uterus, where it is perhaps aided by the intense vasoconstriction (Hertting & Schiefthaler, 1963), or else the escaping transmitter is destroyed much more rapidly than in the spleen. Even if one doubled these figures on the assumption that recovery might have been as low as 50%, the quantities remain a fraction of those which earlier work by Mann & West (1951) had led one to expect. These authors examined the nature of sympathin released by the non-pregnant cat uterus, but their work was done at a time when the techniques employed had a number of drawbacks, so that comparison with the present results is not very profitable. Even considering only those experiments in which Mann & West had tied off the adrenal glands, the difference cannot be resolved owing to the absence, in the older work, of information on amine content of either arterial blood or control venous samples; among other facts unknown at the time is the long persistence in the circulating blood of very small quantities of catecholamines.

In view of the small amounts of catecholamines appearing in the effluent it is interesting that as little as 0·5 μg adrenaline injected intravenously into a 2 kg cat usually relaxed the uterus; however, as a rule, more than 0·5 μg adrenaline was required to match the effect of supramaximal stimulation of the hypogastric nerve.

The fact that adrenaline appears in the effluent on stimulation of the hypogastric nerves does not necessarily mean that it is manufactured in the uterus. The methylating enzyme phenylethanolamine-*N*-methyltransferase is lacking in the rat uterus (Wurtman, Axelrod & Potter,

1964), and if the same holds for the uterus of the cat one must assume that the adrenaline is supplied from the circulation. Since it is known that sympathetic neurones readily take up circulating catecholamines, an extra-uterine origin of the adrenaline would not be surprising and would perhaps account for the extremely variable proportion in which the two amines appear in the effluent.

Of the drugs which inhibit uptake of catecholamines by the tissue, only dichloroisoprenaline increased the amount of transmitter released into the uterine blood. This may be connected with the fact (Farrant, Harvey & Pennefather, 1964) that this drug inhibits uptake of noradrenaline into the cat uterus. The failure of the other inhibitors to act in the same way may have two reasons: either, though inhibiting uptake in the heart, they have no such effect on the cat's uterus; variations between tissues in this respect have been stressed by Stafford (1963) and by Farrant *et al.* (1964). The alternative is that these drugs, while causing more transmitter to escape from the tissue during the first stimuli, gradually prevent escape altogether when the stimulation is repeated. Such a phenomenon occurs in the perfused spleen of the cat (Blakeley, Brown & Geffen, 1964). If trains of 200 stimuli (at 10/sec) are applied every 10 min, phenoxybenzamine, which prevents uptake in this organ, at first increases the amount of noradrenaline (the 'overflow') entering the effluent, but stops the overflow altogether when eight such trains of stimuli have been administered. At present it is not known whether such observations are valid for, say, desmethylimipramine acting on the cat uterus.

SUMMARY

1. One hypogastric nerve was stimulated electrically in the anaesthetized, eviscerated and adrenalectomized cat, and blood was collected from the ovarian vein. Adrenaline and noradrenaline were estimated in the effluent.

2. Usually small quantities of noradrenaline, and sometimes of adrenaline, were found in arterial blood and in ovarian-vein blood collected before stimulation.

3. In the majority of experiments, stimulation of the hypogastric nerve for periods ranging from 2·5 to 10 min caused a rise in the noradrenaline content of the uterine blood. The appearance of traces of adrenaline was ascertained in seven of the seventeen experiments in which amines were detected during nerve stimulation.

4. The quantity of catecholamines escaping into the circulation was invariably extremely small, the highest (uncorrected) figures being: noradrenaline 5 ng/min, and adrenaline 0·6 ng/min.

5. Of four drugs known to inhibit 'uptake' of catecholamines by heart or spleen only dichloroisoprenaline increased the 'overflow' of amines into the ovarian blood. At most the overflow was doubled.

6. By taking into consideration the relative potency of adrenaline and noradrenaline in each individual uterus it was possible to infer that, in a number of experiments, the motor response to nerve stimulation was essentially due to the noradrenaline released, in a few others to the adrenaline. This does not imply that the adrenaline is formed in the uterine tissue.

7. There appears to be no change in the nature of the transmitter liberated when uterine responses to hypogastric nerve stimulation change from relaxation to contraction as a result of pregnancy.

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