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THE EFFECTS OF FOUR MONOAMINE OXIDASE INHIBITORS ON THE RAT UTERUS

BY

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It has been found (Lindsay, Poulson & Robson, 1961; Poulson & Robson, 1964) that when some monoamine oxidase inhibitors are administered to mice during the first half of pregnancy they interrupt the gestation. That this action is possibly exerted on the central nervous system is shown by the fact that it can be reversed by prolactin or by progesterone. However, a direct effect on the uterus was not completely excluded.

The effect of amine oxidase inhibitors on smooth muscle has been studied by a number of investigators. Benson, Stefko & Roe (1952) reported that iproniazid has a weak spasmolytic effect on the rabbit isolated intestine and a weak antihistaminic effect on the guinea-pig ileum. Zbinden, Randall & Moe (1960), using the rabbit isolated uterus and rat seminal vesicle preparations, found that the action of adrenaline was blocked by phenelzine and by iproniazid. It thus appears that monoamine oxidase inhibitors may have some direct or indirect action on smooth muscle which does not involve the inhibition of the enzyme amine oxidase, and it was thus thought worth while to investigate the pharmacological effects of these compounds on the rat uterus isolated *in vitro*. Accordingly, four of these substances were selected; iproniazid and nialamide which readily interrupt pregnancy, phenelzine, the action of which is weak, and tranylcypromine which has no effect at all on pregnancy (Poulson & Robson, 1963, 1964). The actions of these substances were studied on the pregnant uterus and on uteri in the progestational and oestrous phases.

METHODS

Female albino rats of the Wistar strain, weighing approximately 200 g, were used. The animals were divided into four groups: two of the groups of rats were treated with hormones to induce either the oestrous or the progestational phase in the uterus, a third group was composed of pregnant animals and a fourth was treated with reserpine.

(1) *Oestrous group.* These animals were injected subcutaneously with 2.5 mg/kg of stilboestrol as a single dose 24 hr before the experiment.

(2) *Progestational group.* These rats were ovariectomized by the dorsal route during ether anaesthesia. On the day of operation they were injected with 5.0 mg of progesterone intraperitoneally and 2.5 mg/kg of stilboesterol subcutaneously. On each of the first five postoperative days the animals were injected intraperitoneally with 5.0 mg of progesterone and they were used on the sixth day.

(3) *Pregnant group.* Animals were used on the 20th to 22nd day of pregnancy and in all instances before parturition occurred.

(4) *Group of rats treated with reserpine.* A group of animals were injected intraperitoneally each with 7.5 mg/kg of reserpine on two consecutive days. This dose of reserpine is half that which will deplete the noradrenaline stores in the cat uterus (Burn & Rand, 1960). As reserpine is known to inhibit the oestrous cycle, each rat received 2.5 mg/kg of stilboestrol, subcutaneously, as a single dose on the second day of treatment with reserpine. This ensured that the animals were in the oestrous phase when they were killed on the third day, approximately 18 hr after the second dose of reserpine.

The degree of cornification of the vaginal epithelium was assessed in all animals by examining vaginal smears taken on the day of the experiment.

The animal was killed by a blow on the head and the main vessels of the neck were cut. The entire uterus was removed immediately and each horn was suspended separately in a 25-ml. organ-bath containing mammalian Ringer-Locke solution at 37° C and gassed by a constant stream of air. Recordings were made on a smoked drum with an isotonic lever which was adjusted to have a constant load of 2.0 g and a magnification of 2.5.

Drugs used. These were: iproniazid (Marsilid, Roche), nialamide (Niamid, Pfizer), phenelzine (Nardil, Wander) and tranylcypromine (Parnate, Smith, Kline & French); acetylcholine chloride (Roche), atropine sulphate (B.D.H.), ergotamine tartrate (Sandoz), adrenaline acid tartrate (Burroughs Wellcome), phentolamine (Ciba), pronethalol (I.C.I.), bromolysergic acid diethylamide (BOL, Sandoz) and stilboestrol (Organon); progesterone (Organon) and reserpine (Serpasil, Ciba) were obtained from the manufacturers as oily and aqueous solutions respectively.

Notes on solutions. Iproniazid, phenelzine and tranylcypromine were made up as concentrated acid solutions and stored at 4° C for not more than four days. Immediately before use the solutions were brought to *p*H 7 by the addition of N-sodium bicarbonate solution. All solutions were diluted with Locke solution. Stilboestrol was dissolved in arachis oil.

RESULTS

The rat isolated uterus, suspended in Locke solution, has an intrinsic rhythm which does not differ greatly in either height or frequency of contraction for each stage of the reproductive cycle. The contractions are regular and occur at intervals of 0.5 to 1 min. During any one experiment, which frequently extended over a period of 6 to 7 hr, the pattern of contraction remained constant. There were no marked changes of tone such as occur in isolated preparations of the small intestine.

Three of the amine oxidase inhibitors used, iproniazid, phenelzine and nialamide, inhibited the spontaneous activity of the uterus and sometimes decreased the tone of the muscle. Fig. 1,*a*, *b* and *c* illustrates complete inhibition of the spontaneous activity of the uterus. Reduction of the amplitude occurred within 5 sec of the addition of the drug and recovery of the preparation was usually complete within 1 to 2 min after washing. Relatively high concentrations of these drugs in the organ-bath were necessary before the inhibition occurred. The lowest concentrations of iproniazid and phenelzine which completely inhibited the spontaneous rhythm of the uterus ranged from 10^{-5} to 5×10^{-4} and of nialamide from 10^{-4} to 10^{-3}. Uteri in the progestational phase were more sensitive to iproniazid and phenelzine than were uteri from pregnant animals; there was no difference in sensitivity to nialamide of uteri in different phases. Tranylcypromine, the only non-hydrazide amine oxidase inhibitor used, had a motor effect in a concentration of 10^{-4} (Fig. 1,*d*). Large doses of tranylcypromine (5×10^{-3}) in addition to increasing the frequency of contraction also produced a decrease in amplitude. There was no obvious variation in sensitivity to this drug in uteri obtained from animals in different phases of the cycle. In the reserpinized oestrous animals the effects of the four drugs were the same as in those animals treated with stilboestrol alone. All the amine oxidase inhibitors slightly decreased the motor effect of acetylcholine.

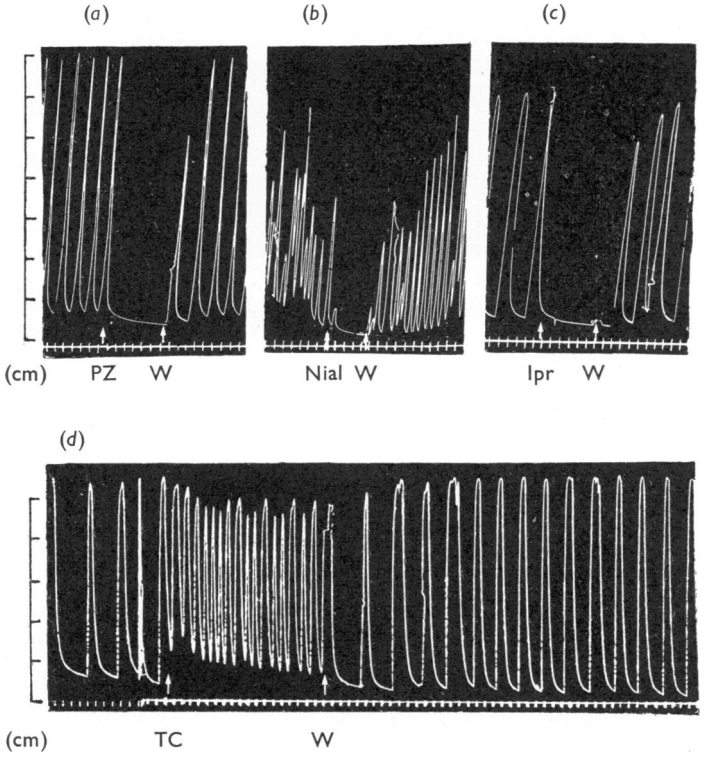

Fig. 1. The effects of four monoamine oxidase inhibitors on the rat uterus. (*a*) Uterus in progestational phase; at the first arrow, phenelzine (PZ, 10^{-5}); (*b*) uterus from animal 22 days pregnant; at the first arrow, nialamide (Nial, 10^{-4}); (*c*) uterus in oestrous phase; at the first arrow, iproniazid (Ipr, 10^{-4}); and (*d*) uterus in oestrous phase; at the first arrow, tranylcypromine (TC, 10^{-4}), which causes a considerable motor effect. W, Washed at least twice. Time marks, 30 sec; ordinate scale, cm on kymograph.

The effects of blocking agents

Pronethalol, when added to the bath in a concentration of 5×10^{-7} (a concentration which prevented the action of adrenaline, 10^{-7}), at least 5 min before the addition of iproniazid, phenelzine or nialamide, completely prevented the inhibitory action of these drugs on the uterus. The blocking effect of pronethalol on the inhibitory action of iproniazid is shown in Fig. 2. It can be seen that pronethalol in this dosage had apparently no direct action on the uterus. Pronethalol was extremely difficult to remove from the preparation and it required frequent washing over a period of 30 to 40 min before a full inhibitory response to the amine oxidase inhibitor could again be obtained. Although the actions of iproniazid, phenelzine and nialamide were prevented by pronethalol, the motor response of the uterus to tranylcypromine remained unaffected in the presence of this drug.

Atropine, in a concentration of 10^{-6}, which is sufficient to prevent the action of acetylcholine but not to affect the intrinsic rhythm, did not in any way affect the actions of the four amine oxidase inhibitors.

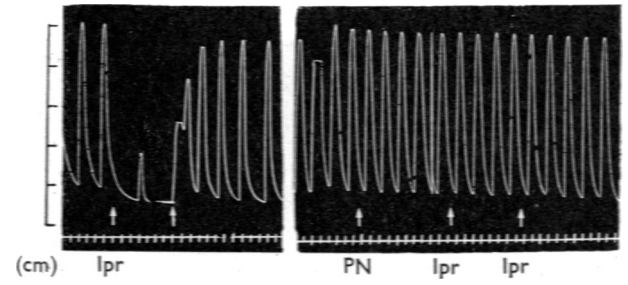

Fig. 2. The effect of pronethalol on the response of the rat uterus to iproniazid. First arrow, iproniazid (Ipr, 2×10^{-4}); second arrow, solution changed; third arrow, pronethalol (PN, 5×10^{-7}); fourth arrow, iproniazid (2×10^{-4}); and fifth arrow, iproniazid (4×10^{-4}). Between the first and second part of the tracing there was an interval of 10 min. Time marks, 30 sec; ordinate scale, cm on the kymograph.

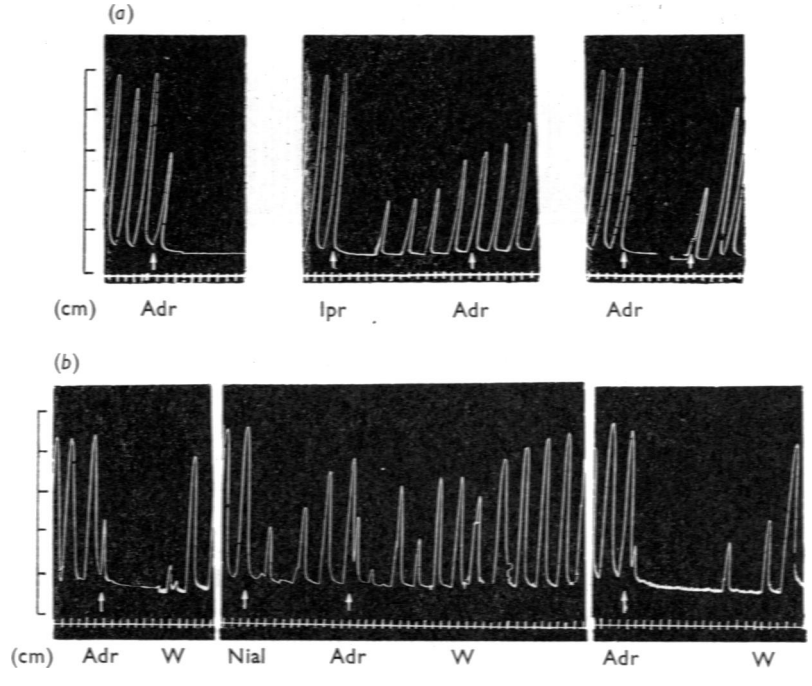

Fig. 3. The actions of iproniazid and nialamide on the response of the rat uterus to adrenaline. Time marks, 30 sec; ordinate scale, cm on the kymograph.

(*a*) Oestrous uterus. First arrow, adrenaline (Adr, 10^{-7}); second arrow, iproniazid (Ipr, 2×10^{-4}); third arrow, adrenaline (10^{-7}), with absence of any inhibition; fourth arrow, adrenaline (10^{-7}); and fifth arrow, solution changed. Between the first and the second parts of the tracing there was an interval of 10 min and between the second and third parts there was an interval of 5 min; during both these intervals the solution was changed at least twice.

(*b*) Oestrous uterus. First arrow, adrenaline (10^{-7}); second arrow, nialamide (Nial, 10^{-4}); third arrow, adrenaline (10^{-7}), with partial inhibition; and fourth arrow, adrenaline (10^{-7}). Between the first and second parts of the tracing there was an interval of 8 min, and between the second and third parts 66 min. W, Wash.

Two sympathetic α-receptor blocking agents, phentolamine (10^{-7}) and ergotamine (10^{-7}), were also ineffective in modifying the actions of these drugs. Bromolysergic acid diethylamide (10^{-7}), which prevents the action of 5-hydroxytryptamine (10^{-8}), did not block the motor effect of tranylcypromine.

Adrenergic blocking effects of the amine oxidase inhibitors

The rat uterus is very sensitive to the action of adrenaline which is inhibitory throughout the oestrous cycle and pregnancy. Since iproniazid, nialamide and phenelzine have an action similar to that of adrenaline, and therefore may produce an effect at the sympathetic β-receptors, it seemed desirable to investigate whether these amine oxidase inhibitors could in any way modify the response of the rat uterus to adrenaline. Iproniazid (Fig. 3,*a*) and phenelzine, in concentrations which produced some inhibition, completely blocked the action of adrenaline. Nialamide (Fig. 3,*b*) and tranylcypromine only partially blocked the inhibitory action of adrenaline although these drugs were added to the organ-bath in a concentration which produced an effect on the uterus. In one instance phenelzine prevented the action of adrenaline without itself inhibiting the intrinsic rhythm of the uterus. This effect is being further investigated.

DISCUSSION

The four substances investigated are all classified as monoamine oxidase inhibitors, so it seems possible that they might act by preventing the destruction of catechol amines or 5-hydroxytryptamine. In the preliminary experiments it was found that the three hydrazides inhibited the spontaneous contractions of the rat uterus, whereas tranylcypromine had a motor effect. Furthermore, these actions remained unchanged in the reserpinized preparation in which the noradrenaline and 5-hydroxytryptamine stores had been depleted. Since it has been reported that the chromaffin tissue of the uterus is unable to synthesize catechol amines (Hall, 1964), and it was found that amine oxidase inhibitors were still active on the reserpinized (depleted) preparation, it can be concluded that the drugs investigated act on the rat uterus by a mechanism independent of adrenaline and 5-hydroxytryptamine.

The effects of these substances on the rat uterus may be a direct action on the adrenaline or 5-hydroxytryptamine receptors or they may involve other receptors. The fact that the actions of the three inhibitory substances were prevented by pronethalol, which blocks the β-receptors of catechol amines, did suggest that iproniazid, nialamide and phenelzine were acting at the same site as adrenaline, although pronethalol could have an affinity for other receptors on which these substances might act. Tranylcypromine, however, could not have been acting on the 5-hydroxytryptamine receptors as the motor effect produced by it was not blocked by bromolysergic acid diethylamide.

In this present work all four amine oxidase inhibitors prevented the action of adrenaline to some extent. This was partial in the case of nialamide and tranylcypromine and complete with the other two drugs. Iproniazid, phenelzine and nialamide blocked adrenaline only when these were applied in an inhibitory dose, so it would appear that they had some sympathomimetic activity on the very receptors which they blocked. According to Stephenson's (1956) modified receptor theory, these three drugs might be classified as weak partial agonists rather than true antagonists. These drugs possibly act on the sympathetic β-receptors having a high affinity and a low efficacy. It is therefore concluded that

iproniazid, phenelzine and nialamide may act on the β-receptors in the rat uterus. Tranylcypromine has a different action which does not involve the 5-hydroxytryptamine receptors.

Poulson & Robson (1963) showed that iproniazid, phenelzine and nialamide interrupt pregnancy. They suggested that this effect was probably indirect and mediated through the pituitary gland, although a direct effect on the uterus was not wholly excluded. It seems unlikely that the three hydrazides interrupt pregnancy by a direct action on the uterus as they all have an inhibitory action. On the other hand, tranylcypromine, the action of which on the rat uterus is motor and which therefore may have been significant in this respect, does not interrupt pregnancy. This work therefore supports the hypothesis that the interruption of pregnancy by the amine oxidase inhibitors occurs by way of a mechanism remote from the uterus.

SUMMARY

1. The mechanism by which pregnancy is interrupted by iproniazid, nialamide and phenelzine was studied on the rat uterus *in vitro*. The effects of tranylcypromine, which does not interrupt gestation, were studied in a similar manner.

2. Two groups of rats were first treated with the appropriate hormone so that the uteri were in the progestational or oestrous state. A third group was 20 to 22 days pregnant and a fourth group was reserpinized.

3. Iproniazid, nialamide and phenelzine inhibited the intrinsic rhythm of the rat isolated uterus preparation whereas tranylcypromine had a motor effect. These effects were similar in all stages of the reproductive cycle and in the reserpinized animal.

4. The inhibiting action of iproniazid, nialamide and phenelzine was prevented by the sympathetic β-blocking agent, pronethalol.

5. All four substances prevented, to some extent at least, the inhibitory action of adrenaline.

6. It is concluded that iproniazid, phenelzine and nialamide may affect sympathetic β-receptors in the rat uterus, and that their actions on the uterus play no part in the interruption of pregnancy.

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