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PREVENTION BY ANTAGONISTS OF THE TOXIC ACTION OF 5-HYDROXYTRYPTAMINE ON PREGNANCY

BY

EVELYN POULSON AND J. M. ROBSON

From the Department of Pharmacology, Guy's Hospital Medical School, London, S.E.1

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As 5-hydroxytryptamine can interrupt pregnancy in all its stages in mice and as pregnancy can be protected from the action of 5-hydroxytryptamine by either progesterone or by prolactin only in early (days 1 to 6) but not in late pregnancy (day 14), the present experiments with two antagonists of 5-hydroxytryptamine, namely methysergide and cyproheptadine, were carried out. Each compound antagonized the action of 5-hydroxytryptamine in late pregnancy. The toxic effects produced by 5-hydroxytryptamine are probably due to a direct action on the placental circulation which is prevented by the antagonists. In early pregnancy, methysergide prevented the effect of 5-hydroxytryptamine, which is believed then to act on the central nervous system, interfering with luteal activity. Cyproheptadine alone interrupted early pregnancy, an action which was prevented by progesterone.

Lindsay, Poulson & Robson (1963) have shown that 5-hydroxytryptamine can interrupt pregnancy in mice at all stages of gestation. Pregnancy could be protected from the toxic effects of 5-hydroxytryptamine by the simultaneous administration of either progesterone or prolactin during the early stages (days 1 to 6), but this protection was not apparent in the late (day 14) stages of pregnancy. It was suggested that, in early pregnancy, 5-hydroxytryptamine interfered with the activity of the corpus luteum probably through some central action on the pituitary gland, while later in pregnancy 5-hydroxytryptamine exerted a direct toxic effect on the uterine contents.

The present experiments were planned to show whether the toxic effects of 5-hydroxytryptamine on pregnancy were modified by antagonists of 5-hydroxytryptamine and whether more information could be obtained about the mode of action of 5-hydroxytryptamine, especially in late pregnancy.

METHODS

Two antagonists of 5-hydroxytryptamine were used, namely methysergide and cyproheptadine. Methysergide is a methylated derivative of lysergic acid. A 1% solution was prepared by dissolving 100 mg of the bimaleate salt in 1 ml. of N-methane sulphonic acid and adding 5% glucose solution to make 10 ml. Cyproheptadine, a potent antagonist of histamine and of 5-hydroxytryptamine (Stone, Wenger, Ludden, Stavorski & Ross, 1961), was administered as a solution in distilled water.

5-Hydroxytryptamine was administered as the creatinine sulphate dissolved in distilled water, and the doses given are of the salt. Progesterone was given as a solution in oil. All experiments were carried out using albino mice of known fertility bred in the Animal House, Guy's Hospital Medical School, and pregnancy was dated from the finding of the vaginal plug.

RESULTS

Effects of antagonists of 5-hydroxytryptamine in late pregnancy

The first investigations were carried out using methysergide. Five groups of pregnant mice were taken.

Mice of the first (control) group received 1 mg of 5-hydroxytryptamine in a single subcutaneous injection on the afternoon of day 14. Those of the second group received 25 μg of methysergide subcutaneously 30 min before the injection of 1 mg of 5-hydroxytryptamine on the afternoon of day 14. Mice of the third group received progesterone, four subcutaneous doses of 1 mg each, given on the afternoon of day 13 and at 12-hourly intervals subsequently, and 1 mg of 5-hydroxytryptamine on the afternoon of day 14. The fourth group was treated as the third but in addition the mice received 25 μg of methysergide 30 min before the injection of 5-hydroxytryptamine. Mice of the fifth (control) group received 25 μg of methysergide only on day 14.

All mice were killed on the afternoon of day 15, about 24 hr after the injection of 5-hydroxytryptamine, and the uterine contents examined. The results are shown in Table 1. Methysergide almost completely protected the litters against the toxic effects of 5-hydroxytryptamine and no further protection was afforded by the additional administration of progesterone.

TABLE 1

PROTECTIVE EFFECT OF METHYSERGIDE AGAINST THE DELETERIOUS ACTION OF 5-HYDROXYTRYPTAMINE IN PREGNANCY ON THE 14TH DAY

No additional protection was seen when progesterone was also administered. See text for doses and times of administration of drugs. * Including one complete abortion. † In four mice

Treatment	No. of pregnant mice	No. of litters affected	No. of foetuses (live/total)
5-Hydroxytryptamine	5	5*	5/24†
5-Hydroxytryptamine +methysergide	8	2	50/53
5-Hydroxytryptamine +progesterone	8	8	12/58
5-Hydroxytryptamine +progesterone +methysergide	16	5	96/110
Methysergide	2	0	6/6

Experiments using cyproheptadine were carried out using three groups of pregnant mice. Mice of the first (control) group received 1 mg of 5-hydroxytryptamine subcutaneously on the afternoon of day 14. Those of the second group received cyproheptadine, three subcutaneous injections of 0.2 mg each, given on the morning of day 14 and at 12-hourly intervals subsequently and 1 mg of 5-hydroxytryptamine on the afternoon of day 14 at the same time as the second injection of cyproheptadine. Mice of the third group received cyproheptadine only, given as for the second group.

All mice were killed on day 15, 24 hr after the injection of 5-hydroxytryptamine, and the uterine contents examined. The results are shown in Table 2. Cypro-

TABLE 2

THE PROTECTIVE EFFECT OF CYPROHEPTADINE AGAINST THE DELETERIOUS ACTION OF 5-HYDROXYTRYPTAMINE IN PREGNANCY ON THE 14TH DAY

See text for doses and times of administration of drugs

Treatment	No. of pregnant mice	No. of litters affected	No. of foetuses (live/total)
5-Hydroxytryptamine	2	2	1/15
5-Hydroxytryptamine +cyproheptadine	10	2	70/72
Cyproheptadine	2	0	14/14

heptadine afforded virtually complete protection against 5-hydroxytryptamine. The two dead foetuses found in two litters may not be significant, as this number of deaths is sometimes found in litters of untreated animals.

Effects of antagonists of 5-hydroxytryptamine in early pregnancy

Since a single dose of 5-hydroxytryptamine will not interfere with a large proportion of the gestations, six daily doses of 5-hydroxytryptamine and of the antagonist were used. Five groups of mice having vaginal plugs were taken.

Mice of the first group received 5-hydroxytryptamine, 1 mg/day given as single subcutaneous injections in the afternoons on days 1 to 6 of pregnancy. Mice of the second group received methysergide, 25 μg given as a single subcutaneous injection 30 min before the injection of each dose of 5-hydroxytryptamine on days 1 to 6 of pregnancy. Mice of the third and fourth groups received cyproheptadine, 0.4 and 0.6 mg/day respectively. Each dose was given as one subcutaneous injection in the morning and one in the afternoon, the second 30 min before the injection of 1 mg of 5-hydroxytryptamine on days 1 to 6. Mice of the fifth group received 0.4 mg/day of cyproheptadine alone, in two subcutaneous injections given in the mornings and in the afternoons of days 1 to 6.

All animals were killed on day 14 and the uterine contents examined. The results are shown in Table 3 and are expressed as the number of normal litters found in the number of mice with vaginal plugs used in each group. Simultaneous control studies showed that about 65 to 70% of mice found with vaginal plugs became pregnant.

Methysergide antagonized the toxic action of 5-hydroxytryptamine, there being twenty normal litters out of thirty-nine mice, the remaining mice showing no sign of conception. No antagonism was shown by either dose of cyproheptadine, but when the fifth group was examined it was found that cyproheptadine itself had interrupted pregnancy.

A further experiment was done to determine whether the toxic effect of cyproheptadine would be prevented by progesterone. Mice of one group received 0.4 mg/day of cyproheptadine in two subcutaneous injections on days 1 to 6 of pregnancy. In addition all animals received progesterone (2 mg/day) given in two subcutaneous injections on day 1 and until *post-mortem* on day 14. The injections of progesterone (on days 1 to 6) were given simultaneously with the cyproheptadine.

TABLE 3

EFFECT OF 5-HYDROXYTRYPTAMINE AND ITS ANTAGONISTS IN THE EARLY STAGES OF PREGNANCY

The dose of 5-hydroxytryptamine was 1 mg/day. All drugs were given daily on days 1 to 6. Methysergide was given in a daily dose of 25 μg, 30 min before 5-hydroxytryptamine, in the combined experiment. Cyproheptadine was given in two daily doses (total of 0·4 or 0·6 mg/day), and in the combined experiments the first dose was given in the morning and the second in the afternoon 30 min before the injection of 5-hydroxytryptamine. The dose of progesterone, given with cyproheptadine in the last group, was 2 mg/day (1 mg morning and evening) subcutaneously

Treatment	No. of mice showing normal litters/vaginal plugs
5-Hydroxytryptamine	2/17
5-Hydroxytryptamine+ methysergide	20/39
5-Hydroxytryptamine+ cyproheptadine (0·4 mg)	0/23
5-Hydroxytryptamine+ cyproheptadine (0·6 mg)	0/17
Cyproheptadine (0·4 mg)	1/14
Cyproheptadine (0·4 mg)+ progesterone	9/16

At *post-mortem*, nine of sixteen mice had normal live litters, the remaining seven mice having no sign in the uteri of conception (Table 3).

DISCUSSION

The results show that both antagonists of 5-hydroxytryptamine investigated, methysergide and cyproheptadine, antagonized the toxic effects which 5-hydroxytryptamine produces when given in the later stages of pregnancy in mice. There is good evidence that these toxic effects are due to a direct action of the 5-hydroxytryptamine on the uterine contents, probably by interfering with the supply of blood and nutrient substances to the placenta and foetus (Robson & Sullivan, 1963). Since these antagonists counteract the vascular effect of 5-hydroxytryptamine (Doepfner & Cerletti, 1958; Stone *et al.*, 1961), the present findings agree with our conception of the mode of action of 5-hydroxytryptamine at this stage of pregnancy.

The results in early pregnancy, however, raise new problems. We believe that 5-hydroxytryptamine produces its deleterious effect at this stage by interfering with the luteotrophic activity of the pituitary gland, leading to a deficient secretion of progesterone which is essential for the maintenance of pregnancy. This effect may be produced by an action on neurones in the brain or, alternatively, by an action on the vascular supply to some part of the brain, for example, the hypothalamus or even the pituitary gland. As 5-hydroxytryptamine can inhibit gonadotrophic production in both immature and mature female mice without affecting the production of growth hormone (Robson & Botros, 1961), it is unlikely that the drug produces its effect by interfering with the vascular supply to the pituitary gland, for this effect could hardly be so specific as to affect the gonadotrophin-secreting cells and not those responsible for the secretion of growth hormone. The prevention by methysergide of the toxic action of 5-hydroxytryptamine at this early stage of pregnancy shows that the antagonists can prevent the effect of 5-hydroxytryptamine exerted on the central nervous system, though it does not, at present, help to localize more accurately the site of action of the drug.

The results with cyproheptadine in early pregnancy show that this compound, like 5-hydroxytryptamine and like various inhibitors of amine oxidase (Poulson & Robson, 1963), can interfere with the luteal secretion necessary for the maintenance of pregnancy. Further investigation of this particular action is necessary.

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