ON THE MECHANISM OF ACTION OF INTRA-AMNIOTIC HYPERTONIC SALINE TREATMENT IN RABBITS

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Summary. A study was made into the effects of hypertonic saline upon the intra-uterine pressure in non-pregnant and pregnant rabbits. In non-pregnant rabbits intra-luminal administration of hypertonic saline caused an almost immediate transient increase in amplitude and frequency of uterine pressure cycles, a response which was abolished by prior treatment with progesterone. In pregnant rabbits, provided all placentae were damaged by the treatment, intra-amniotic administration of 20% NaCl resulted in abortion of all foetuses in 56 ± 7.3 hr SE. If one conceptus escaped damage pregnancy was not interrupted. Furthermore, treatment with 25 μ g/day oestradiol propionate prevented abortion. Of the procedures carried out, only dislocation of the placentae also caused abortion, whereas pregnancy was not terminated by extra-amniotic administration of hypertonic saline, by cutting the umbilical cords (i.e. causing foetal death), nor by the intra-amniotic administration of normal (0.9%) saline. The abortion produced by both intra-amniotic hypertonic saline and dislocation of the placentae could be prevented by oestrogen administration, but not if ovariectomy were combined with placental dislocation. From these results, it is concluded that intra-amniotic hypertonic saline induced abortion in rabbits by interrupting the luteotrophic function of the placenta which resulted in loss of the luteal function essential for the defence of pregnancy.

INTRODUCTION

The instillation of hypertonic saline into the amniotic sac is used as a method of terminating pregnancy in women, although the mechanism by which this procedure induces abortion is disputed. Csapo (1961) suggested that the abortion is produced by a reduction in progesterone secretion consequent upon structural damage to the placenta caused by the saline. However, claims that extensive necrosis of the placenta follows intra-amniotic hypertonic saline administration (Csapo & Lloyd-Jacob, 1962; Bengtsson & Csapo, 1962; Straus,

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1963; Wynn, 1965; Friedrich, personal communication) have been contested (Christie, Anderson, Turnbull & Beck, 1966). Furthermore, whereas the steroid chemical analyses of Wiest, Kerenyi & Csapo (1966) and Wiest (1967) document that a fall in blood-progesterone occurs following intra-amniotic saline, those of Short, Wagner, Fuchs & Fuchs (1965), Kerr, Roy, Harkness, Short & Baird (1966) and Klopper, Turnbull & Anderson (1966) do not support this hypothesis.

An alternative suggestion that abortion is precipitated by a direct action of saline upon the myometrium, has been advanced by King, Friedman & Steer (1964) and Kerr *et al.* (1966). Other possible mechanisms such as the release of oxytocin through stimulation of the supra-optic nucleus by an elevated blood-sodium concentration, have been reviewed recently (Fuchs, 1967).

Rabbits offered unique advantages for a study of the mechanism of saline-induced abortion. The evolution of uterine activity in pregnant rabbits has been extensively described (Csapo & Takeda, 1965; Fuchs, 1964; Porter & Schofield, 1966; Ogata & Csapo, unpublished data). Furthermore, the rabbit, being polytocus, has more than one amniotic sac, and is dependent upon an extra-uterine (corpus luteum) as well as an intra-uterine (placenta) endocrine organ for the maintenance of pregnancy. These features permitted the control of values which would have been impossible in the human female.

MATERIALS AND METHODS

Non-pregnant rabbits

In a group of ten, non-pregnant New Zealand White rabbits (3.5 to 4.5 kg) laparotomies were carried out aseptically under pentobarbitone (100 to 150 mg i.v.) and ether anaesthesia. Finger-cot balloons of 13-ml capacity, unstretched and attached to polyethylene tubes were inserted, by way of an incision in the vaginal wall and through the cervix into the uterine horns. Balloons were placed in both horns in four animals and in one horn in six animals. Perforated catheters were introduced into the uterine cavity together with the balloons, to permit the intra-uterine administration of saline (Porter, 1968). Each balloon-and-catheter was anchored in the uterus by means of a suture which did not occlude the uterine lumen. The animals were ovariectomized to standardize their endocrine states. The laparotomy wound was closed and the connecting tubes and catheters passed subcutaneously to the back of the neck, brought to the exterior and anchored.

The recording balloons in all animals were filled with water to a volume of 2 to 10 ml (depending upon the size of the uterus) to stretch the myometrium, and thereby induce high myometrial activity (25 to 85 mm Hg intra-uterine pressure).

Each animal was given an injection of 2.0 to 5.0 ml (depending upon the size of the uterus) of 10% NaCl solution into the uterine lumen through the perforated catheter. (10% NaCl was used in an attempt to compensate for the dilution which occurs in the amniotic fluid when 20% NaCl is injected into the amniotic sacs of pregnant rabbits.) In two animals the injection was performed 18 hr after treatment with 5 mg progesterone (intra-muscularly) to

suppress the high activity. Intra-uterine pressure was recorded throughout the experiments by means of Sanborn pressure transducers (No. 267 BC) and pen recorders (No. 321). The records obtained were analysed for changes in the mean amplitude of the active pressures (AP) (i.e. the cyclic pressure changes in excess of 10 mm Hg superimposed upon the resting pressures).

Pregnant rabbits

A group of fifty-one pregnant (25 to 27 days) New Zealand White rabbits was studied. One rabbit, i.e. the 'monitor' rabbit, in each experimental group was equipped with a small (1.5-ml capacity) balloon inserted between the foetal membranes and the endometrium through an incision in the uterine wall of the lower segment. Intra-uterine pressure was recorded as described above.

Experiment I. Intra-amniotic saline. In twenty-eight rabbits all conceptuses were injected with 2.5 to 5.0 ml saline. These rabbits were divided into two groups:

(a) Hypertonic saline: Twenty-two rabbits (experimental) received intra-amniotic injections of hypertonic saline (20%). Of these, five were given oestradiol propionate (25 µg/day) following saline treatment for 3 days. Pilot experiments had shown that the administration of more than 30 ml of 20% NaCl to rabbits frequently resulted in death. Therefore, in six rabbits where the litter size exceeded six or seven, it was reduced by emptying one uterine horn.

(b) Isotonic saline: Six (control) rabbits received intra-amniotic injections of isotonic (0.9%) saline. In three animals with litters exceeding six, one uterine horn was emptied, as a control for the procedure carried out in group (a).

Experiment II. Placental dislocation. In twelve rabbits, all placentae were dislocated by pressure between finger and thumb during laparotomy. In three, bilateral ovariectomy was also performed. Oestrogen therapy (25 µg oestradiol propionate daily s.c. for 4 days) was instituted after surgery in the ovariectomized rabbits and in four in which only placental dislocation had been performed.

Experiment III. Extra-amniotic saline. Six rabbits were equipped with perforated catheters, similar to those used in non-pregnant animals. The catheters were inserted, through a small incision in the uterine wall, between the foetal membranes and the endometrium throughout the length of each horn. Twenty-four hours after surgery hypertonic saline (20% NaCl) was injected through the catheters, into each uterine horn (2.5 to 5.0 ml/conceptus).

Experiment IV. Cutting the umbilical cords. In five rabbits, small incisions were made in the uterine wall over each conceptus and the umbilical cords severed.

Animals in all experiments were repeatedly observed and, after deliveries, the foetuses were examined and weighed. The monitor animals, equipped with recording balloons, were used to obtain an indication of the intra-uterine pressure changes which followed the various procedures, and of the responses to a test dose of 50 m.u. oxytocin (Syntocinon) injected intravenously at various intervals.

Histology

Specimens of uterus (two) and placenta (eight) were obtained from animals in Expts. I, III and IV. They were fixed in buffered formalin at 4° C, sectioned at 5 μ , and stained with haematoxylin and eosin. In addition, the conceptuses in one horn of a rabbit were treated with hypertonic saline, while those in the other horn received an equal volume of physiological saline. The animal was killed 24 hr after treatment and specimens of placenta were obtained from the two horns. These were fixed in Bouin's fixative and prepared for histological examination as described above.

RESULTS

Non-pregnant rabbits

In non-pregnant rabbits the intra-luminal instillation of hypertonic saline caused an increase in both the active pressure (AP) and the frequency of pressure cycles, within 15 min of administration (Pl. 1, Fig. 1). This increase in activity reached a maximum in about 30 min and subsequently declined to pre-treatment values in 50 to 60 min. The AP remained at the pre-treatment level for at least 24 hr after recovery. No response was elicited by the injection of a similar volume of isotonic saline.

The increase in AP was often accompanied by a rise in the resting pressure (five out of eight cases). In four cases there was an increase in the number of irregularly shaped pressure cycles recorded.

In the animals treated with progesterone the administration of hypertonic saline produced only a slight increase in resting pressure, upon which were superimposed a few pressure cycles of less than 10 mm Hg (Pl. 1, Fig. 2).

Pregnant rabbits

Experiment I. Intra-amniotic saline. Of the sixteen rabbits which only received intra-amniotic injections of hypertonic saline, nine aborted all foetuses, dead, in an average of 56 hr (± 7.3 hr S.E.) after treatment (Text-fig. 1). The tracing of intra-uterine pressure from the monitor rabbit in this group (Pl. 2, Fig. 3), shows an initial response to the saline injection and surgical interference, in the form of a slight increase (less than 10 mm Hg) in AP, which diminished within 8 hr. No distinct oxytocin response was detected at 0 and 8 hr. After 24 hr, the AP had increased to about 15 mm Hg and the oxytocin response was marked.

Five rabbits failed to abort and either delivered at term spontaneously or were autopsied. This group maintained pregnancy for an average of 127 hr (± 23.5 hr) (Text-fig. 1) despite the interruption of two pregnancies, shortly before term, by autopsy. In all cases in this group, at least one living foetus was delivered or found *in utero* at autopsy.

The two remaining rabbits treated with hypertonic saline alone, each aborted only one foetus, at 50 and 64 hr after treatment, respectively. Neither delivered additional foetuses despite attempts to induce delivery at term with doses of up to 1.5 units of oxytocin, intravenously. At autopsy, both animals had grossly abnormal uteri, with areas of necrosis, possibly caused by leakage

FIG. 2

FIG. 1. Record of intra-uterine pressure from a non-pregnant ovariectomized rabbit. 5-ml balloons in each horn. 5 ml $\times$ 10% NaCl injected intra-luminally into experimental horn. Control horn: no treatment.
FIG. 2. As FIG. 1, but saline injection performed 18 hr after progesterone (5 mg, i.m.) treatment.

FIG. 1

FIG. 3

FIG. 4

Fig. 3. Record of intra-uterine pressure from a pregnant rabbit (26 days). 1-ml balloon. 5 ml $\times$ 20% NaCl administered intra-amniotically to each conceptus (total 6). Recordings at 0, 8 and 24 hr after treatment. Uterine response to test dose of 50 m.u. oxytocin (i.v.) in right-hand records. Animal aborted in response to oxytocin at 24 hr.

Fig. 4. Pregnant rabbit (27 days). 1-ml balloon. 5 ml $\times$ 20% NaCl administered intra-amniotically to each conceptus (total 5). 25 μ g oestradiol (s.c.)/day. Recordings at 0, 48 and 144 hr after treatment. Responses to test dose of 50 m.u. oxytocin (i.v.) at 48 and 144 hr. No abortion. All foetuses *in situ*, at autopsy at 148 hr.

Fig. 6. Pregnant rabbit (26 days). 1-ml balloon. All placentae dislocated (total 6). 25 μ g oestradiol (s.c.)/day. Recordings at 0, 24, 72, 120 and 168 hr. Responses to test dose of 50 m.u. oxytocin (i.v.) at 72, 120 and 168 hr. No abortion. All foetuses *in situ* at autopsy at 180 hr.

Fig. 5

Fig. 5. Pregnant rabbit (27 days). 1-ml balloon. All placentae dislocated (total 8). Recordings at 0, 18 and 24 hr. Aborted in 31 hr.

Fig. 6

FIG. 7

FIG. 7. Pregnant rabbit (27 days). 1-ml balloon. All placentae dislocated (total 6) and ovaries removed. 25 μ g oestradiol (s.c.)/day. Recordings at 0, 3, 23 and 24 hr. Response to test dose of 50 m.u. oxytocin (i.v.) at 24 hr culminated in delivery (F).

FIG. 8

FIG. 8. Pregnant rabbit (27 days). 1-ml balloon. 35 ml $\times$ 20% NaCl (i.e. total 7 conceptuses) instilled extra-amniotically into the uterine horns (20 ml, right horn, 15 ml, left horn). Recordings at 0, 1, 48 and 72 hr. Responses to test dose of oxytocin (i.v.) at 48 and 72 hr, the latter culminated in delivery.

of hypertonic saline over the serosal surface. These two cases are considered as abnormal and their results disregarded.

The six rabbits treated with hypertonic saline and subsequently given oestrogen injections, maintained pregnancy to term, or beyond (i.e. for an average of $129.6 \text{ hr} \pm 10.7 \text{ hr}$ after saline treatment). Intra-uterine pressure of the monitor rabbit of this group, increased slightly after surgery, but subsided within 48 hr (Pl. 2, Fig. 4). Slight responses to oxytocin were recorded up to 144 hr but these did not culminate in delivery. At autopsy it was found that conceptuses of these six animals were separated by annular constrictions of the uterus.

TEXT-FIG. 1. Summary of data. *Data supplied by courtesy Ogata & Csapo (unpublished). Oestradiol ($25 \mu\text{g/day}$) withdrawn at •; progesterone (5 mg/day) withdrawn at o. Closed columns: all deliveries completed; open columns: some pregnancies interrupted by autopsies.

The six animals which received intra-amniotic injections of normal saline, maintained pregnancy until term (i.e. for $158 \text{ hr} \pm 12.25 \text{ hr}$ S.E. after treatment). Thirty-three foetuses were born alive out of a total of forty-nine.

Experiment II. Placental dislocation. The five rabbits subjected to dislocation of the placentae alone, and the three rabbits in which placental dislocation and ovariectomy were performed, aborted in 31 hr ($\pm 4.1 \text{ hr}$ S.E.) and 38 hr ($\pm 7.9 \text{ hr}$ S.E.), respectively. Tracings from monitor rabbits from both of these categories show that intra-uterine pressure increased rapidly following surgery, and reached 20 mm Hg, AP, within 24 hr (Pl. 3, Fig. 5 and Pl. 4, Fig. 7). In the ovariectomized monitor rabbit (Pl. 4, Fig. 7), the response to oxytocin at 24 hr culminated in delivery.

The four animals treated with oestradiol propionate after placental dislocation did not abort (Text-fig. 1). Autopsy, performed on average 150 hr (± 13.5 hr S.E.) after surgery, confirmed that all foetuses had been retained, macerated, *in utero*, and were separated by annular constrictions of the uterus. The pressure tracing from the monitor animal showed that no significant uterine activity evolved, even as late as 168 hr after placental dislocation, and that responses to oxytocin were negligible (Pl. 3, Fig. 6).

Experiment III. Extra-amniotic saline. The six rabbits in this experiment maintained pregnancy until term (Text-fig. 1), i.e. on average for 126 hr (± 17.5 hr S.E.) after surgery. In all cases, several living foetuses (twenty-two out of a total of forty-six) were delivered or found at autopsy. Pressure tracings from the monitor animal showed only a slight response to hypertonic saline treatment within 1 hr of instillation and this subsided within 24 hr. Distinct but small responses to oxytocin were elicited at 24 and 48 hr, and, in this case, the response at 72 hr culminated in delivery (Pl. 4, Fig. 8).

Experiment IV. Cutting the umbilical cords. The five animals in this experiment delivered near to term (Text-fig. 1), on average 102 hr (± 11.5 hr S.E.) after surgery. Examination of all foetuses at delivery confirmed that cord section had been successful.

Statistical analysis

The numerical data of all experiments were subjected to an analysis of variance (Snedecor, 1956). It was found that the mean treatment-delivery intervals of the following groups: the abortion group of Expt. I and the placental dislocation only and placental dislocation+ovariectomy groups of Expt. II were significantly shorter than those of all other groups, at the 5% level.

Histological examinations

Specimens of placenta taken from the animals in Expt. I, and from conceptuses treated 24 hr previously with hypertonic saline, revealed extensive areas of necrosis in the labyrinth (Pl. 5, Fig. 9). There was marked loss of basophilia, and of cellular detail. Sections of placenta from animals in Expt. III were either normal in appearance, or showed evidence of necrosis. The normal samples were obtained from conceptuses with living foetuses, whereas those with evidence of necrosis were from conceptuses with dead foetuses. Specimens of placenta obtained 24 hr after treatment with normal saline were nearly normal in appearance (Pl. 5, Fig. 10). A specimen from an animal in Expt. IV contained areas of necrosis in the labyrinth, as well as pockets of nearly normal cells. This specimen was obtained at delivery, whereas all others examined were taken at autopsy before delivery had occurred.

DISCUSSION

The experiments on non-pregnant animals demonstrate that the intra-uterine administration of hypertonic saline to animals exhibiting a high uterine AP (i.e. stretch-induced) causes a transient increase in myometrial activity. This

PLATE 5

FIG. 9. Section of placental labyrinth from a rabbit in Expt. Ia treated with intra-amniotic hypertonic saline. Tissue obtained at autopsy of the animal during abortion. H. & E. $\times 200$.

FIG. 10. Section of placental labyrinth from a rabbit in Expt. III. Placenta was obtained at autopsy from a conceptus containing a living foetus. H. & E. $\times 200$.

(Facing p. 438)

observation is in agreement with the findings of Wagner (1966a). It is significant that the myometrium recovered quickly (in 1 hr on average) and completely from the effect of saline, and that saline had virtually no effect in progesterone-treated rabbits. An increase in resting pressure, indicative of contracture, and an irregularity in the shape of the pressure cycles were observed in the majority of tracings. These findings suggest that the myometrial response to hypertonic saline was one of dysfunction, which is consistent with the observation of Hendricks & Tucker (1959) that hypertonic saline causes progressive deterioration of human and rat myometrial activity *in vitro*, where the hypertonicity remained constant. The transient abnormality of the pressure cycles in the present experiments suggests that, *in vivo*, the hypertonicity was rapidly eliminated.

The salient feature of the experiments on pregnant rabbits is that abortion was induced only when hypertonic saline was injected successfully into every amniotic sac or when all placentae were dislocated, whereas all other procedures were ineffective. In considering what factors might have caused these abortions it is also necessary to explain the failure of the other procedures to interrupt pregnancy. Possible causative factors include:

A. Extra-uterine: (1) a systemic effect (e.g. increased blood-sodium level) causing a release of oxytocin; (2) surgical trauma.

B. Intra-uterine: (1) direct effect of saline upon the myometrium; (2) foetal death; (3) increase in volume of amniotic fluid; (4) destruction of the placentae.

Considering each possibility in turn, it would seem that release of oxytocin (A.1) is unlikely. Neither extra-amniotic saline treatment (Expt. III), nor the intra-amniotic saline injections, which failed to kill all the foetuses (Expt. Ia), induced abortion, yet all animals in these groups received doses of saline which, when all foetuses died (Expt. Ia), caused abortion.

Surgical trauma (A.2) as the cause of abortion may be eliminated, since animals in Expt. Ib, treated with isotonic saline, underwent similar trauma to those which aborted after hypertonic saline treatment (Expt. Ia). Indeed, the animals treated with isotonic saline (Expt. Ib) delivered 66% of their foetuses alive, a figure which, in the circumstances, compares favourably with the 76% observed by Shanklin (1966) in normal deliveries.

It is also improbable that a direct effect of saline upon the myometrium was responsible for the abortions (B.1), since, after both intra- and extra-amniotic saline, only a slight increase in AP was recorded immediately, which subsided within 24 hr. In contrast, the activity which culminated in abortion was characterized by a gradual evolution, resembling that previously (Csapo & Takeda, 1965) and presently (Expt. II) recorded after dislocation of the placentae, although more delayed. It is therefore concluded that the induction of uterine activity by intra-amniotic injection of hypertonic saline in pregnant rabbits and the transient stimulation of AP by intraluminal instillation in non-pregnant rabbits are different phenomena.

Abortion could not be attributed to foetal death alone (B.2), since cutting the umbilical cords failed to cause abortion. This is consistent with veterinary (Arthur, 1964) and obstetric (Csapo, 1967) experience that foetal death *in utero* does not induce abortion promptly as does, for example, *abruptio placentae*.

The possibility that abortion was due to an increase in uterine volume alone is unlikely (B.3). Although an increase in amniotic fluid volume probably occurs following saline treatment, due to the hypertonicity, a similar increase would have occurred in those animals in Expt. Ia where one or more foetuses survived the treatment and where abortion did not ensue. The activation of the myometrium by stretch is an acute phenomenon, and is sustained only if the stretch (i.e. the volume) is maintained (Csapo, Takeda & Wood, 1963). However, in view of the reports of King *et al.* (1964) and Wagner (1966b) that the hypertonicity of the amniotic fluid, after saline treatment, is rapidly reduced, it seems unlikely that the increased volume in the present experiments would have persisted long enough (i.e. for 56 hr in Expt. Ia) to account for the abortions.

From the above considerations, it is evident that the first five hypotheses (A.1 and 2, B.1, 2 and 3) fail to explain fully the results of the present experiments. In considering whether placental destruction (B.4) might be responsible for the abortions, several factors should be noted. The results of Expts. II and IV indicate that the integrity of the placenta only is essential for the maintenance of pregnancy since destruction of the whole foeto-placental unit (Expt. II) induces abortion, whereas foetal death alone (Expt. IV) does not. It is assumed that cutting the umbilical cords did not cause the immediate destruction of the placentae, since removal of the foetuses is followed by the delivery of the placentae at term (Klein, 1933; Newton, 1935; Kirsch, 1938; Schofield, 1960) and pockets of nearly normal tissue were found in placentae obtained at term from animals in Expt. IV.

Since oestrogen therapy maintained pregnancy after placental dislocation alone, but not if ovariectomy was performed as well, it can be concluded that the effect of oestrogen, in partially replacing placental function, is mediated through the ovary and not directly upon the myometrium. This observation is consistent with those of Westman & Jacobsohn (1937), Robson (1937) and Heckel & Allen (1939) that the life of the corpus luteum (and thus pregnancy) is prolonged in rabbits by oestrogen therapy. Furthermore, the present data support the conclusions of several workers (Klein, 1933; Mayer & Canivenc, 1953; Schofield, 1960; Greep, 1941; Allen & Heckel, 1939; Csapo & Lloyd-Jacob, 1961) that the integrity of the placenta is essential for the maintenance of the corpus luteum in the rabbit whereas that of the foetus is not.

Since oestrogen was capable not only of supporting pregnancy after placental dislocation but also after intra-amniotic saline treatment, it would appear that the abortions induced in the present experiments were due largely to a disruption of a luteotrophic function of the placenta. This hypothesis can explain how extra-amniotic saline failed to induce abortion and how, when at least one foetus (and placenta) survived, intra-amniotic treatment was unsuccessful. (The survival of foetuses after intra-amniotic treatment was probably due to the saline being injected extra-amniotically in error.) Also the long injection-abortion time (56 hr) in Expt. Ia, in contrast to the prompt abortions (31 hr) in Expt. II, may indicate that saline requires a definite period of time to cause placental necrosis, whereas placental dislocation causes an abrupt cessation of the luteotrophic function.

The present data support the conclusion of Csapo (1961) that the successful instillation of hypertonic saline into the amniotic sac initiates the evolution of uterine activity (and hence abortion) by causing a partial withdrawal of progesterone consequent upon necrosis of the placenta.

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