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SIDE EFFECTS AND MECHANISM OF  
ACTION OF PROSTAGLANDINS

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## I. . INTRODUCTION

The consultants made a visit to Dr. A. Csapo at Washington University, St. Louis, Missouri, on October 1 and 2, 1979, in order to evaluate the contract AID has with the University to study the "Side Effects and Mechanism of Action of Prostaglandins."

## II. SUMMARY OF FINDINGS AND RECOMMENDATIONS

It is recommended that support for this laboratory and program be continued. The strength of this program derives from Dr. Csapo's experience in the physiology of uterine contraction and the productivity this laboratory has documented in both testing in animals and development of clinical protocols to examine compounds which induce progesterone withdrawal. More than 50 publications have been submitted or published, all in relatively good journals. It is recommended that the current level of support be maintained and possibly adjusted to cover additional salary components of the in-house staff of Washington University.

Support of this research has led to the elucidation of the mechanism underlying initiation and maintenance of uterine contractions. A particularly important contribution of this laboratory has been the description of progesterone withdrawal and its relevance to the induction of early labor and normal labor at term. These mechanisms have contributed to an increased understanding of the basis of prostaglandin induction of abortion. This information has not led to better compounds for clinical use but screening of compounds is still in progress. Delivery system development was to be undertaken in the contract, but effort has been limited and confined mainly to an evaluation of recommended systems from the manufacturers. Of particular significance with respect to progress has been the conduct of clinical studies. This effort has permitted an evaluation of efficacy of compounds and also has led to new and important basic information.

Performance of this contract with progesterone synthesis inhibitors has been limited to an examination of the compound isoxazole. This work was done in animals and has led to greater understanding of uterine function, but it is doubtful that this approach will lead to a clinically useful compound. There has been some progress in development of prostaglandin analogues with fewer side effects. This research is encouraging and it is possible that a self administrable prostaglandin for early abortion or early menstrual regulation may eventually be found.

### III. FINDINGS

1. What has been the performance of the project in discovering the mechanisms underlying the initiation and maintenance of uterine contraction in the human as well as other animals?

A particularly strong aspect of the research being carried on by Dr. Csapo has been on the mechanism of initiation and maintenance of uterine contractility. This contribution has been original in the realm of gathering new information but more importantly, in the development of a unifying hypothesis. This laboratory continues to develop new insights into the role of progesterone on inhibition of uterine contractility and of equal if not greater importance, the abrogation of progesterone action which then leads to induction of cyclic uterine contractility. In the human it has been difficult to reconcile the clear blocking action of progesterone on myometrium which is seen in laboratory animals. This laboratory was the first to show such an effect and has forwarded new evidence that a similar phenomenon occurs in the human. At present, there is no clear decrease of any great magnitude in circulating progesterone at term delivery in the human and the mechanism of initiation of labor in the human remains an enigma. Dr. Csapo's lab, however, has provided clear evidence for such a role for progesterone following lutectomy early in the pregnancy. In these studies removal of progesterone by lutectomy led to development of uterine contractions with oxytocin treatment but was absent if progesterone replacement was given. The direct demonstration of Csapo and colleagues that the concentration of progesterone in the myometrium adjacent to the placenta was much higher than in areas of the myometrium distant from the placenta is laudable. This observation gains more credence from the additional finding that spontaneous uterine activity was lower at sites near the placenta than at sites distant from the placenta. The latter data was obtained in the rat and it would be highly relevant to determine if a similar situation exists in the human.

Other elements in the mechanism underlying initiation and maintenance of uterine contraction of which progesterone withdrawal is an integral part of the overall unifying hypothesis includes:

(1) Vasoconstriction of the uteral-placental blood vessels.

At term this is accomplished by the increasing frequency of spontaneous uterine contractions due to stretching of the myometrium as a consequence of fetal growth. The frequency of spontaneous contraction occurs due to the proportionately greater growth of the uterus compared to the placenta resulting in a physiological progesterone withdrawal. With prostaglandin treatment Csapo and colleagues show the direct vasoconstrictive action of the drug on the uterine vessels.

(2) Anoxia; vasoconstriction of uterine produces anoxia.

(3) Sustained contracture; anoxia per se is well known to induce a uterine contracture and prostaglandin (generally accepted by many to be the endogenous

fetus. The decrease in progesterone levels precedes evolution of the cyclic increases in uterine pressure which are necessary for the impending delivery.

At normal delivery no profound decrease in progesterone concentrations in peripheral blood is seen prior to the induction of labor although a small decrease in progesterone occurs. The role of progesterone withdrawal for term delivery is unknown and no clear evidence for such a mechanism exists in the human at this time. Professor Csapo believes that labor at term is due to a disproportionate growth of uterine myometrial mass with respect to the source of progesterone (the placental mass). Early in gestation, the relatively high content of progesterone produced by the placenta and the relatively high placental mass with respect to the balance of the uterine myometrial tissue, permits progesterone to effectively block uterine contractility. However, with increasing growth of the fetus, the uterine myometrial mass increases and eventually overcomes the blocking effect of the progesterone produced by the placental tissue. Csapo suggests that stretch per se may be the signal for the development of labor at term. There is some evidence for this hypothesis seen in the clinic in that excess fetal growth results in premature delivery (for example diabetes). Also, the introduction of a substantial volume of isotonic saline into the uterine lumen prior to term will result in evolution of uterine contractility and delivery.

3. Has understanding these mechanisms contributed to the process of arriving at better compounds for clinical use?

Understanding the mechanisms of progesterone withdrawal and induction of myometrial contractility has not contributed directly to the development of better compounds. The present generation of compounds all depend upon induction of uterine contractility as a means for inducing the response. This response appears to be due to an early induction of uterine contraction along with uterine vasoconstriction in early gestation and in mid-trimester pregnancy. This is believed to be the mechanism for induction of progesterone withdrawal. Stimulation of endogenous prostaglandin later acts to induce cyclic intrauterine contractions. It is generally believed that the side effects of the prostaglandin analogues are due to indiscriminate stimulation of smooth muscle activity in areas outside of the uterus. Probably the second generation of compounds may induce progesterone withdrawal which permits production of endogenous prostaglandin leading to cyclic uterine contractions. Dr. Csapo is aware of this possibility and indeed has done preliminary work in this area.

Nonetheless the recognized time dependent changes in the requirements necessary for evolution of cyclical intrauterine pressure by prostaglandin has been of value. In this regard, Dr. Csapo is presently formulating plans to examine a delivery system of prostaglandins with vaginal pessories which can provide a quick release in conjunction with a sustained slower release. The rationale behind this approach is that the quick release pessory may induce the rapid contracture and vasoconstriction and the slow release pessory will provide prostaglandin to aid in the development of the cyclic uterine pressure required for delivery. No evidence is presently available to indicate whether such a formulation would be more effective than the present methods of either intrauterine administration or vaginal pessory.

scientific observation and is an important and positive function which has contributed to the overall high quality of achievement. The clinical studies involving endocrine consequents of luteotomy are unique as a human model system and have been highly productive in elucidating basic mechanisms. The inclusion of the Beta sub-unit determination for HCG would enhance studies in early pregnancy.

7. What has been the performance of the project in the use of progesterone synthesis inhibitors as a means of fertility regulation?

As described in Article 1 of Statement of Work this objective was not outlined in the original proposal but arose from a report of the activity of the compound (isoxazole) from Sterling-Winthrop. Dr. Csapo provided considerable evidence on the effect of this compound in laboratory animals on reducing progesterone and inducing abortion in the rat. As such it has provided useful information for testing the hypothesis that progesterone withdrawal is a prerequisite for abortion. In addition, some evidence in the non-human primate indicates that this compound may be selective on ovarian progesterone biosynthesis but have relatively little effect on adrenal steroidogenesis. In regard to animal testing, the performance of the laboratory in testing this compound has been excellent. However, the problem of potential side effects in the human has not been addressed. This compound is a cyanoketone derivative (a drug with known teratogenic effects in the rat) and shows no such tissue specificity in non-primates.

8. What is the future potential of progesterone synthesis inhibitors?

In view of this reviewer the future potential of progesterone synthesis inhibitors as a means for fertility control in the human is questionable. The Sterling-Winthrop compound isoxazol is a derivative of an older compound cyanoketone and both compounds appear to act as inhibitors of 3-beta-1 dehydrogenase activity. Since progesterone is a parent steroid to the formation of all remaining steroids in both the adrenal and ovary, the potential for side effects of such a compound is high. However, such compounds are useful in the laboratory for the testing of mechanisms and as such may be useful tools to the identification of alternate physiological mechanisms or drugs.

9. What have been the by-products of this research?

By-products of the current project have been fruitful in better defining basic mechanisms of action in term and premature labor, dysfunctional labor, spontaneous abortion and dysmenorrhea. The currently planned collaborative investigation of nuclear progesterone receptors is both innovative and possible implications are of great clinical significance. Although the present project is focused upon abortion, the investigator, by his own words is a "mechanism man" both in theory and practice. It is this valuable asset which has allowed the investigator to expand his findings beyond the stated scope of the project.

#### IV. RECOMMENDATIONS

1. The strengths of this proposal lie in two areas. First, Dr. Csapo has been working in the area of control of uterine contractility for many years and as a consequence has developed techniques and procedures which permit this laboratory to continue as one of the outstanding centers in the world in this area. Secondly, this laboratory has wide experience in development of clinical protocols to test compounds which induce progesterone withdrawal and lead to the development of uterine contractions. In particular this laboratory has been central to the demonstration that prostaglandins in mid-trimester and in first trimester abortion produce their effects by induction of progesterone withdrawal. At one time this was open to some criticism but leaders in the area now appear to agree with the hypothesis of Dr. Csapo. This important finding will probably have great consequences in the development of any new compound with this type of action or use. Because of these contributions and proven performance support for this laboratory and program should be continued.

2. This has been a productive research program in terms of both gaining clinical expertise and in drug development, and in furthering our knowledge with respect to the latter, more than 50 publications have been submitted and these have been in relatively good journals. The cost effectiveness of the personnel has been outstanding. Indeed it is because of Dr. Csapo's prestige and worldwide reputation that he is able to obtain the clinical collaboration necessary for the conduct of these studies. As such the payments to such clinicians in countries outside the U.S. is modest but not excessively so. With regard to radioimmunoassay costs commercial assays inherently are more expensive than identical assays done within the laboratory. At Yale the cost analysis for radioimmunoassay determinations on human plasma are in the neighborhood of \$20 per sample.

The staff working in-house at Washington University seem to be underpaid. This is particularly the case for the M.D. staff. Present guidelines from the NIH are to pay a basic salary of \$10,000 with a degree and an additional \$500 component per year for every year of experience beyond the M.D. or Ph.D. This generally places postdoctoral salaries in the neighborhood of \$14,000 per year.

For the present level of activities it is recommended that the current level of support be maintained and possibly adjusted to cover additional salary components of the in-house staff at Washington University.