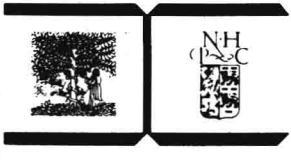
Recent Advances in Reproduction and Regulation of Fertility

G. P. Talwar
Editor

RECENT ADVANCES IN REPRODUCTION AND REGULATION OF FERTILITY

Proceedings of the Symposium on Recent Advances in Reproduction and Regulation of Fertility held in New Delhi, India, October 24-28, 1978.

Editor
G.P. TALWAR



1979

ELSEVIER/NORTH-HOLLAND BIOMEDICAL PRESS
AMSTERDAM · NEW YORK · OXFORD

ISBN Elsevier/North-Holland: 0-444-80123-5

Published by:
Elsevier/North-Holland Biomedical Press
335 Jan van Galenstraat, PO Box 211
Amsterdam, The Netherlands

Sole distributors for the USA and Canada:
Elsevier North-Holland Inc.
52 Vanderbilt Avenue
New York, N.Y. 10017

Printed in the Netherlands

PREFACE

The world population is expected to reach the six billion mark by the year 2000. In countries like India, the annual increase is 2.1% e.g. about 13 million people, equivalent to the entire population of Australia, are added to the populace every year. The situation is similar in most developing countries of Asia, Africa and Latin America. This may get further accentuated if the decrease in mortality brought about by introduction of immunoprophylaxis and public health programmes is not accompanied by an equivalent reduction in birth rates.

Futurologists consider population control as a major problem of global concern. There are two facets of this issue. The increase is not evenly spread, in some countries the growth rate is high while in others the numbers are not only static but negative trends are perceptible. No doubt socio-economic factors are important in motivation of small family norms. There are however, also biomedical aspects. Sterility is rampant side by side with high incidence of fertility. Both demand a better understanding of the reproductive processes and development of better, safer and more acceptable methods for promotion or prevention of fertility.

This book is based on papers presented in a Symposium organized by the Indian National Science Academy with support from the Family Planning Foundation of India, the Rockefeller Foundation and the World Health Organization. The objectives of the Symposium were : (a) To take stock of the progress that has taken place in recent years in understanding of the reproductive mechanisms and (b) to identify the improvements and innovations either made or being made in contraceptive technology, which could be expected to be available to the users in the next decade.

The Symposium succeeded in getting together a number of leading investigators in the field. Both male and female reproductive system are covered, and many new developments of pertinent importance are presented. The range is wide, from neuro-endocrine mechanisms to gametogenesis, fallopian tube physiology, fertilization, early embryonic development, regulation of corpus luteum function and implantation. The implications of the synthesis and secretion of foetal proteins such as hCG, by tumours and extraplacental cells is discussed. Of interest are the characteristics of teratocarcinomas which share antigens with developing embryo. The mechanisms by which the conceptus escapes immunological rejection have been comprehensively reviewed. The

current status of male and female antigens of promise (LDH-x: zona pellucida, hCG, LHRH) for developing anti-fertility vaccines has been brought out by those actively involved in this research. Wherever feasible, applied research to develop new methods has been dovetailed with discussion of basic processes. These include the nasal sprays, the non-occlusive intra-vasal metallic devices, non-steroidal anti-implantation compounds; and use of luteolytic agents for synchronizing estrus in farm animals to increase the efficiency of artificial insemination methods for breeding. The remarkable properties of a small molecular weight substance extracted from human seminal plasma to improve the motility and survival of sperms from a number of species are also described.

I am much indebted to my co-workers, students and administrative staff members, in particular Drs. C. Das, Brian D'Monte, S.K. Dubey, S.N.S. Hanjan, Mr. S. Ramakrishnan, Mr. R. Naz, Mr. M.R. Nigam and Mrs. A. Bhatia, for their invaluable help in organization of the Symposium.

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ACKNOWLEDGEMENTS

I gratefully acknowledge the following organizations without whose sponsorship this Symposium would not have been possible :

The Indian National Science Academy
The Family Planning Foundation of India
The Council of Scientific and Industrial Research
The Rockefeller Foundation and
The World Health Organization

The Symposium was an official satellite symposium of the Fifth International Congress on Hormone Steroids.

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THE NEURAL CONTROL OF THE MENSTRUAL CYCLE

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The menstrual cycle of the rhesus monkey (Macaca mulatta) is remarkably similar, at least in a qualitative sense, to that of human female. It has a duration of 28 days, on the average, and ovulation occurs approximately 36 hours after a midcycle surge of luteinizing hormone (LH) and follicle stimulation hormone (FSH). The time course of serum gonadotropin concentrations throughout the menstrual cycle may be regarded as being the resultant of tonic FSH and LH secretion interrupted by a preovulatory discharge of these polypeptides from the pituitary gland. This pattern of gonadotropin secretion is governed by the negative and positive feedback actions of ovarian estrogens. The tonic secretion of both LH and FSH is controlled by a simple negative feedback loop wherein estradiol-17β is the primary ovarian component. Progesterone does not appear to play a significant physiological role in this regard, in sharp contrast to its important negative feedback action in other species. Follicular development and estrogen secretion proceed in response to tonic gonadotropin stimulation during the follicular phase of the menstrual cycle until serum estradiol concentrations exceed a threshold of approximately 200 pg/ml for 36 hours or more. The positive feedback action of the steroid then comes into effect resulting in initiation of the preovulatory discharge of FSH and LH (1).

That these control systems can be subserved by the medial basal hypothalamus (MBH)-hypophysial apparatus has been suggested by experiments in which the hypothalamic deafferentation technique devised by Halasz and co-workers for the rat (2,3) was applied to the rhesus monkey (4). The completeness of the surgical disconnections of the MBH in female rhesus monkeys was evidenced by microscopic examinations of the lesions and by various physiological criteria (4,5,6). The MBH "islands" produced in these animals included the median eminence and arcuate nucleus, and portions of the ventromedial nuclei and of the mammillary bodies. The preoptic area as well as the suprachiasmatic and supraoptic nuclei were excluded by the cuts.

Basal FSH and LH levels were not severely altered in either intact or ovariectomized animals subjected to this procedure and the circhoral, pulsatile gonadotropin discharges characteristic of gonadectomized monkeys (7) remained

fully evident. In addition, the negative feedback inhibition of gonadotropin secretion by estradiol was easily demonstrable in these monkeys. Unexpectedly, however, the administration of estradiol benzoate to monkeys subjected to either complete or anterior deafferentation of the MBH induced gonadotropin surges indistinguishable from those produced in non-lesioned animals, and in some of the deafferented animals, normal spontaneous ovulatory menstrual cycles were observed (4). These findings differ dramatically from those in the rat, wherein either complete or anterior deafferentation of the MBH interrupts the ovarian cycle, by interfering with the preovulatory discharge of the gonadotropins. This latter species normally requires connections from the preoptic-anterior hypothalamic area to the MBH for the transmission of a neural signal, coupled to the diurnal light-dark cycle, which initiates the preovulatory gonadotropin surge at a given time of day (see 8). This seemingly fundamental difference between the effects of MBH deafferentation in the monkey and in the rat, coupled with the observation of Norman et al. (9), that radiofrequency lesions in the preoptic-anterior hypothalamic area abolished the positive feedback action of estrogen on gonadotropin secretion in the rhesus monkey, raised the possibility that the surgical isolation of the MBH described in the monkey by Krey et al. (4), may not have been functionally complete. Nerve regeneration not demonstrable by ordinary histological techniques could not be ruled out nor could the transport of a neurotransmitter, or luteinizing hormone releasing hormone (LRH) itself, to the disconnected MBH either by diffusion across the surgical scar or by way of the cerebrospinal fluid. The relatively high concentrations of LRH reported in the region of the organum vasculosum of the lamina terminalis in the rhesus monkey (10) was of particular concern in this regard. We have directly addressed these possibilities by aspirating all neural tissue anterior and dorsal to the optic chiasm (11). Removal of the lamina terminalis, preoptic area and anterior hypothalamus and of the suprachiasmatic, as well as portions of the paraventricular, dorsal medial and ventral medial nuclei did not interfere with estrogen induced discharges of LH and FSH which were strikingly similar in their time courses and magnitudes to those observed in animals with intact nervous systems following estradiol benzoate administration.

It may be concluded from the foregoing that, in the rhesus monkey, the central components of the feedback loops which control the tonic mode of gonadotropin secretion, as well as the preovulatory surge of FSH and LH, are resident within the MBH-hypophysial unit. The conclusion that the monkey, unlike the rat, does not appear to require a signal from the preoptic-anterior hypothalamic area for the initiation of the preovulatory gonadotropin surge is consonant with the findings that

such surges, whether spontaneous (12) or induced (13), are not coupled to the diurnal light-dark cycle and that neuroactive drugs such as pentobarbital, reserpine or phenoxybenzamine, which block the diurnal neural signal required for ovulation in the rat (14), have no effect on estrogen induced gonadotropin surges in the rhesus monkey (1). Similarly, adult male rhesus monkeys can respond to the positive feedback action of estradiol with an LH surge (15), whereas similarly treated male rats cannot (16), presumably because, in the latter, neonatal androgens have abolished the transmission of the diurnal signal from the anterior hypothalamus to the MBH (17).

The negative and positive feedback actions of estradiol in the control of tonic and surge secretion of the gonadotropic hormones in the rhesus monkey may be at the neural level and there modulate GnRH release, at the level of the pituitary gland and there control gonadotropin release directly or by altering the sensitivity of the gonadotrophs to ambient levels of GnRH, or any combination of these three alternatives. Indirect evidence can be adduced in favor of all these possibilities (1). In any event, the secretion of GnRH by the MBH is limiting in the control of gonadotropin secretion. Large, bilateral radiofrequency lesions in the MBH of ovariectomized rhesus monkeys, extending from the optic chiasm to the mammillary bodies and including the dorsal aspect of the median eminence, lead to a prompt decline in serum FSH and LH concentrations to immeasurable levels and abolish the positive feedback action of estradiol (19). Similarly, the intravenous administration to ovariectomized monkeys of antisera to synthetic LRH also results in a rapid reduction in serum gonadotropin levels which remain depressed as long as the circulating antibody titer remains elevated (18). The rapidity of the response to the antiserum suggests that the sustained production of the releasing hormone is required for the elevated gonadotropin secretion characteristic of these animals.

That the arcuate nucleus is the portion of the MBH responsible for the control of LRH release is suggested by the finding that large radiofrequency lesions in the MBH which spare this area have little or no effect on tonic gonadotropin secretion or on estrogen induced LH and FSH release while smaller lesions, restricted primarily to the region of the arcuate nucleus, result in a decline of serum gonadotropins to undetectable levels as early as 48 hours after the placement of the lesions. The administration of estradiol benzoate several days later fails to elicit a gonadotropin surge (19). Attempts to reestablish sustained gonadotropin secretion in such animals by the continuous infusion of exogenous GnRH, however, were unsuccessful. Although impressive increases in serum gonadotropin concentrations could be observed for a day or two following the initiation of the decapeptide infusions, the animals became refractory to continued treatment,

serum gonadotropin returning to undetectable levels, regardless of the magnitude of the hypophysiotropic stimulus (19,20,21). When, however, an intermittant GnRH replacement regimen which mimicked the circhoral mode of gonadotropin secretion was instituted (20,22), preoperative serum levels of LH and FSH were reestablished (20,21). These unexpected observations have led us to the conclusion that the pattern of hypophysiotropic stimulation is an important new dimension in the neural control of gonadotropin secretion (21). In fact, we have recently demonstrated that relatively small changes in the frequency of exogenous GnRH administration to monkeys with MBH lesions profoundly influences, not only the magnitude of the resulting gonadotropin response, but also the FSH:LH ratio in the circulation (23). The latter phenomenon is attributable to the major differences in the metabolic clearance rates of the two glycoprotein hormones.

Using ovariectomized rhesus monkeys bearing hypothalamic lesions which abolish gonadotropin secretion and in which pre-lesion gonadotropin levels were reestablished by the chronic, intermittant administration of GnRH, we have re-explored the sites of the feedback actions of estrogen (20). In these preparations, which were devoid of an endogenous source of GnRH, estrogen exhibited its characteristic negative (20,22) and positive (20) feedback effects thus permitting the conclusion that both these actions can be at the level of the pituitary gland. That estradiol can initiate gonadotropin surges by acting directly on the gonadotrophs has also been shown in monkeys with transected pituitary stalks (24).

The above findings have led us to formulate the hypothesis that GnRH, while an essential component of the neuroendocrine control system which governs the menstrual cycle of the rhesus monkeys is, nevertheless, a permissive one, primary control being exerted by the rising and ebbing tides of estradiol in the plasma. We have recently tested this hypothesis in otherwise intact rhesus monkeys bearing hypothalamic lesions which have abolished gonadotropin secretion. When these animals were subjected to a GnRH replacement regimen consisting of hourly pulses, the majority eventually exhibited, normal ovulatory menstrual cycles 29 to 33 days in duration (25). At the completion of the experiments these animals were ovariectomized but their serum gonadotropin levels remained undetectable, even in the face of large doses of estradiol benzoate, suggesting that reorganization of the neural component of the control system had not taken place in the course of the study. The experimental design employed was essentially identical to that previously described for lesioned, ovariectomized animals (20).

From all of the foregoing, we have constructed a model of the neuroendocrine control system which directs the ovarian cycle of the rhesus monkey. It consists of a neuronal element in the region of the arcuate nucleus which, in the absence of

any extrahypothalamic neural input, discharges a bolus of GnRH approximately once every hour. In the intact system the frequency of this "circhoral GnRH clock" is undoubtedly modulated by higher centers with resultant changes in mean circulating gonadotropin levels and in FSH:LH ratios. The primary control of the basic pattern of gonadotropin secretion, i.e. tonic secretion interrupted once every 28 days by a preovulatory goandotropin surge, is provided by the negative and positive feedback actions of estrogen acting at the level of the pituitary gonadotrophs which require intermittant GnRH stimulation for their functional integrity. The duration of the cycle in this model, is determined by the duration of follicular development in response to basal, intermittant gonadotropin secretion and the preprogramed functional lifespan of the corpus luteum (26) during which follicular development is inhibited.

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