Blockade of Ovulation by Melatonin

Numerous publications have recently demonstrated that the pineal gland exerts an inhibitory activity on the reproductive system of both male and female animals[1-6]. This inhibition seems to be exerted through a certain number of substances synthesized by the gland, i.e. melatonin (5-methoxy-N-acetyl-tryptamine), 5-methoxytryptophol, 5-hydroxytryptophol, and serotonin (5-hydroxytryptamine)[3-6].

Preliminary evidence seems to indicate that the pineal gland intervenes also in the control of ovulation. Pinealectomy has been shown to enhance the ovulatory response following the administration of gonadotropins to immature rats[7-10] while melatonin and 5-methoxytryptophol appear to inhibit copulation-induced ovulation in rabbits[11,12].

In a series of experiments we have tried to obtain a direct proof of the participation of pineal melatonin in the control of spontaneous ovulation in the rat. This substance has been previously shown to be able to inhibit the secretion of pituitary luteinizing hormone (LH) which is probably the major ovulating gonadotropin in this species[13,14].

In the present experiments melatonin was injected into one of the lateral ventricles of the brain around the so-called 'critical period' of the day of proestrus during which ovulating amounts of LH are released from the pituitary[15-17].

Material and methods. Adult female Sprague-Dawley rats were implanted permanently with a cannula which enabled us to inject melatonin into one of the lateral ventricles of the brain without anesthesia, thus avoiding the wellknown blocking effect of anesthetics on ovulation[15,16]. After implantation of the cannula, the estrous cycles of these rats were carefully verified. Only animals showing at least 2 regular four-day cycles were utilized. The animals were killed the day following the injection of melatonin, and ovulation was determined by counting the ova in the Fallopian tubes under a dissecting microscope.

Since the half-life of melatonin is only about 30 min[18], and the 'critical period' of the day of proestrus lasts about 2 h, a single injection of melatonin was considered inadequate to block ovulation. Melatonin, dissolved in methanol and diluted with saline to a final concentration of 20 to 100 µg/20 µl, was therefore injected every hour for 4 or 5 times beginning approximately 60 min before the 'critical period' which, in our strain of rats, and with the lighting conditions of our animal quarter (14 h light, 10 h darkness) goes from 14.00 h to 16.00 h. 19 rats received in this way a total amount of melatonin varying from 100 to 500 µg per rat. In a control experiment melatonin was injected s.c.

Results and discussion. The results, shown in the Table, indicate that ovulation was completely blocked in 7 rats out of 19. In the 12 rats that ovulated, and which constitute the 63% of treated animals, a mean number of 5 ova was found. 8 rats were injected intraventricularly with saline solution following the same schedule used for melatonin. All ovulated, and the mean number of ova was 11.7.

A group of 11 rats was injected s.c. with a total amount of 800 to 1200 µg/rat of melatonin given in 4 or 5 times around the 'critical period'. All of them ovulated, and the mean number of ova was not significantly different from that of the saline-treated animals.

The Figure shows that an inverse relationship exists between the total amounts of intraventricularly injected melatonin and the mean number of tubal ova. It is interesting to notice that in a certain number of rats ovulation was completely blocked even with the lowest amounts of melatonin.

These results indicate that melatonin is able to block spontaneous ovulation in the rat either completely or partially following intraventricular injections. The compound is ineffective when given s.c. even if higher doses are used. The negative data obtained when melatonin was administered s.c. seem to confirm the hypothesis

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Effect on ovulation of intraventricular (ivt) and s.c. injections of melatonin during the 'critical period' in mature rats

Groups	No. of rats treated	No. of rats ovulating	Rats ovulating (%)	Average of ova ovulating rat	No. of rats not ovulating
Saline (ivt)	8	8	100	11.7 ± 0.1[a]	0
Melatonin (ivt) 100–500 µg/rat	19	12	63	5.0 ± 0.7[b]	7
Melatonin (s.c.) 800–1200 µg/rat	11	11	100	10.1 ± 0.9	0

[a] Means $\pm$ S.E. [b] $P < 0.0005$ vs saline.

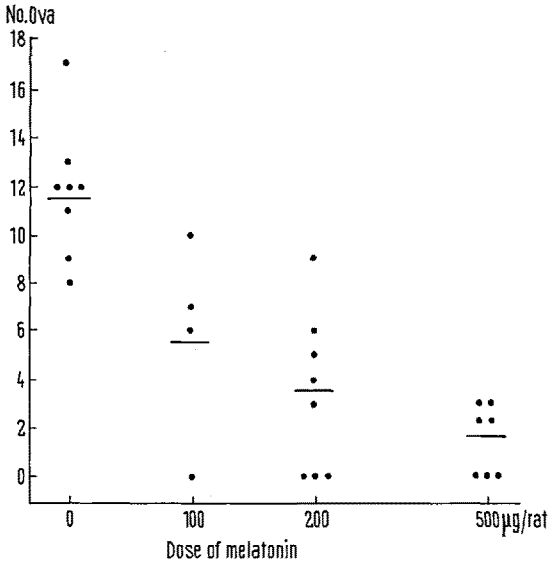
that the physiological way of secretion and transport of melatonin is probably through the cerebro-spinal fluid[19].

Several hypotheses may be advanced in order to explain the inhibitory activity of melatonin on ovulation. The blocking action of this substance might be due to its ability to inhibit LH secretion by acting on specific brain receptors[13, 20, 21].

Recent data have also shown that melatonin, when injected i.v. in new-born chickens[22] or implanted in small amounts into the preoptic area of cats[23] can produce sleep. In addition, intraventricularly and i.p. administered melatonin is able to prolong the sleeping effect of pentobarbital[24, 25]. The sedative action of melatonin has also been documented by electrophysiological techniques, which show the appearance of slow and high voltage waves on the EEG and a decrease of the periods of paradoxical sleep[23, 26–28]. On the basis of these data one might postulate that melatonin, acting as a sedative, could block ovulation through an inhibitory effect exerted on neuronal pathways involved in the control of the ovulatory processes. According to this hypothesis the mode of action of melatonin would not be too different from that of barbiturates and other sedatives.

Recently it has been reported that progesterone of adrenal origin may facilitate the release of the ovulatory surge of LH[29]. On the other hand, the secretion of progesterone from the adrenal gland is controlled by pituitary ACTH[30–32]. Since melatonin has been recently reported to inhibit ACTH secretion, following intraventricular injections[33], a third hypothesis one might formulate is that melatonin inhibits ovulation by preventing the release of progesterone from the adrenal gland.

Finally, one might also hypothesize that melatonin blocks ovulation because of its ability to increase brain stores of serotonin[34]. KORDON et al.[35] have recently found that the blockade of ovulation induced by the administration of monoaminooxidase inhibitors is specifically linked to increased brain serotonin levels. It is quite possible that these 4 proposed mechanisms might coexist, melatonin inhibiting ovulation through a combination of its effects.



Number of ova found in Fallopian tube after intraventricular injection of increasing amounts of melatonin.

Résumé. L'injection de mélatonine dans un des ventricules latéraux du cerveau de rattes adultes, pendant la «période critique» du pro-œstrus, inhibe l'ovulation spontanée, entraînant une diminution significative soit du nombre de rattes qui ovulent, soit du nombre moyen d'œufs tubaires.

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Identity of a Claimed Growth-Promoting Factor

BOZOVIĆ, BOSTRÖM and BOZOVIĆ[1] recently claimed that they had from calf muscle isolated a growth-promoting factor which stimulated the transport of amino acids into isolated rat diaphragm and which furthermore promoted protein synthesis. The conclusions were based on experiments with ^{14}C-AIB and ^{14}C-leucine. It was furthermore suggested that this factor was dependent on growth hormone, thus agreeing with KOSTYO's[2] hypothesis concerning a polypeptide or protein first being synthesized by the growth hormone and that this substance could be responsible for the action on the isolated rat muscle. The growth-promoting factor