challenge in these experiments was due to tumor rejection immunity, but passive transfer and specificity experiments would be required to establish that as fact. The resistance we have demonstrated may not be very strong, even in those cohorts which were 100 percent resistant to the challenge with 10^3 cells, but this is the first report of such a high frequency of resistance to LSTRA cell challenge induced by any prior treatment with LSTRA cells.

We do not suggest that these data can be extrapolated directly to every clinical situation. These observations may apply to immunization with tumor cell–adjuvant mixtures, but they may not be of general relevance to the development of immunity following intratumor injection of immune stimulants. The results need confirmation in other laboratories and ought to be extended in this and other animal tumor models. This study has illustrated the potential complexities in the design of rational immunotherapy protocols: When administering immune stimulants, more may not be better; regression of injected lesions may not correlate with systemic immunization against tumor rejection antigens.

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16. We thank D. C. Katsilas for his excellent technical assistance. Supported by PHS contract N01-CB-33891 from the Division of Cancer Biology and Diagnosis, National Cancer Institute, by the Jake Gittlen Memorial Golf Tournament, and by PHS research career development award KO4-CA-70948 to G.L.B.

22 September 1975

Melatonin Inhibition of the Neonatal Pituitary Response to Luteinizing Hormone–Releasing Factor

Abstract. *Neonatal rat anterior pituitary glands treated in organ culture with 1 nanomolar luteinizing hormone–releasing factor (LRF) showed a tenfold increase in medium luteinizing hormone (LH) concentrations over control values. Simultaneous treatment of the glands with 1 nanomolar melatonin significantly reduced the stimulatory effect of LRF on release of LH. This finding indicates that melatonin can act directly on the neonatal pituitary to inhibit the LH response to LRF.*

Numerous studies (*1*) have shown that the antigonadotropic effects of the pineal gland may be mediated in part by melatonin. However, the precise mechanism by which melatonin inhibits reproductive function remains to be resolved. We now report that melatonin can act directly on the pituitary gland to suppress the release of luteinizing hormone (LH) induced by luteinizing hormone–releasing factor (LRF).

The effect of melatonin on the LH response to synthetic LRF (*2*) was studied in organ culture with the use of anterior pituitary glands from 5-day-old female Sprague-Dawley rats. The method of culture used in this study (*3*) is identical to that developed in our laboratory for the culture of pineal glands (*4*). With this system, there is no apparent cellular necrosis in 5-day-old pituitaries incubated for 4 days (*3*).

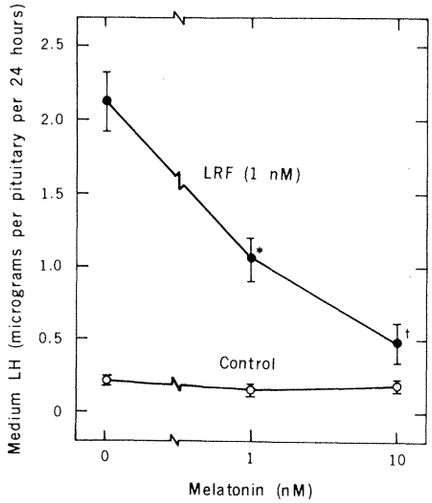

Fig. 1. Effect of melatonin on LRF-induced release of LH. After 24 hours of culture under control conditions, anterior pituitary glands from 5-day-old female rats were treated for 24 hours with control medium, melatonin (1 or 10 n*M*), LRF (1 n*M*), or LRF (1 n*M*) plus melatonin (1 or 10 n*M*). Each point represents the mean response of four anterior pituitaries. Vertical lines represent standard errors. *Significantly less than 1 n*M* LRF and 0 melatonin value; $P < .01$. †Significantly less than 1 n*M* LRF and 0 melatonin value; $P < .001$.

After 24 hours of culture under control conditions, pituitary glands were transferred to fresh medium containing the test substances. Half of the glands were stimulated with LRF (1 n*M*) either alone or in the presence of melatonin (1 or 10 n*M*). The remaining half of the glands served as controls and were incubated either in control medium or in medium containing only melatonin (1 or 10 n*M*). The culture was terminated after a 24-hour treatment period. The LH content of the medium was measured by double antibody radioimmunoassay, in which materials supplied by the Rat Pituitary Hormone Distribution Program of the National Institute of Arthritis, Metabolism, and Digestive Diseases were used. Values are expressed in terms of the reference preparation NIAMDD-Rat-LH-RP-1. Melatonin in culture medium at a concentration of 1 μM does not interfere with the measurement of LH in our assay (*5*). Statistical analysis was made by Student's *t*-test.

As shown in Fig. 1, LRF treatment in the absence of melatonin produced a tenfold increase in the concentrations of LH in the medium over control values. However, simultaneous treatment of the pituitary glands with LRF and 1 n*M* melatonin resulted in a highly significant ($P < .01$) reduction of LH secretion. At 10 n*M*, melatonin suppressed the LRF-induced release of LH to 14 percent of the response obtained with LRF alone. Melatonin had no detectable effect on control concentrations of LH at either of the doses used.

These results indicate that melatonin can act at the pituitary level to suppress

LRF-induced release of LH. Furthermore, this inhibitory effect is evident in vitro at physiologic concentrations. When measured either by bioassay (*6*) or by radioimmunoassay (*7*), concentrations of melatonin in human serum attain values at night of approximately 1 n*M*, a dose which was shown in the present study to inhibit LH release. Other studies in our laboratory (*8*) have demonstrated that the in vitro response to melatonin is rapid, as evidenced by complete suppression of LRF-induced release of LH during a 90-minute incubation period. In addition, specificity of the response in organ culture is indicated by the finding (*8*) that two compounds closely related to melatonin, *N*-acetylserotonin and 5-methoxytryptamine, at concentrations as high at 100 n*M* do not affect the LH response to LRF.

In view of the finding that in the adult rat injection of melatonin into the third ventricle of the brain suppresses LH secretion, whereas injection into a hypophyseal portal vein has no effect on serum LH, Kamberi *et al.* (*9*) have suggested that melatonin inhibits the release of LRF into portal blood. Thus, it appears that melatonin may act both at the hypothalamic level and at the pituitary level to regulate LH secretion. However, their failure to observe suppression of serum LH by melatonin administered directly to the pituitary gland in vivo may be due to the low level of basal LH secretion. It may also be related to the age of the animals. Adult rats were used in their experiments, but neonatal rats were the source of the pituitary glands in our study.

The present finding that LRF stimulation of LH release can be modulated by physiological concentrations of a secretory product of the pineal gland may explain in part the known inhibitory effects of this gland on the reproductive system.

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12 November 1975

Mixed Cultures of Sperm and Leukocytes as a Measure of Histocompatibility in Man

Abstract. *Human peripheral blood leukocyte cultures containing varying numbers of washed fresh sperm were cultured for 4 days. [^{3}H]Thymidine incorporation was used as a measure of lymphocyte transformation. Human sperm cells induce a 4- to 250-fold increase in [^{3}H]thymidine incorporation in allogeneic leukocyte cultures, but no increase was demonstrated in autologous leukocyte cultures. The response was dose-dependent with maximum stimulation obtained at 2×10^6 sperm per milliliter of culture. Seminal plasma was inhibitory in a dose-dependent fashion and as little as 0.2 microliter per 200 microliters of culture was inhibitory. The data indicate that tissues other than leukocytes can express the portion of the major histocompatibility complex responsible for allospecific lymphocyte transformation, and thus may have application in transplantation and reproductive biology.*

The mixed leukocyte culture (MLC) test is widely used as a histocompatibility test in humans and other mammalian species and is recognized as being principally dependent on differences of one genetic region in the major histocompatibility complex (*1*). In recent years, dissociated epidermal cells have also been shown to be capable of inducing lymphocyte transformation and DNA synthesis in allogeneic leukocyte cultures in the rat (*2*), the mouse (*3*), and man (*4*). Epidermal cells are the first cell type not present in peripheral blood that have been shown to be capable of inducing allospecific lymphocyte transformation. We now present data indicating that the sperm present in human ejaculates are also capable of stimulating lymphocyte transformation and DNA synthesis when added to allogeneic but not autologous leukocyte cultures (*5*). Sperm induce levels of lymphocyte transformation similar to that observed with an equivalent number of allogeneic peripheral blood leukocytes.

Fresh sperm were prepared for culture by washing two times with RPMI 1640 medium (Grand Island) that was heated to 38°C, centrifuging at 300*g* for 6 minutes and resuspending in RPMI 1640 to give a final concentration of 1×10^8 sperm per milliliter. Varying portions of the stock sperm suspension were added to leukocyte cultures to yield a final culture concentration of 0.1×10^6 to 5.0×10^6 sperm per milliliter. Leukocytes rich in mononuclear cells were prepared for culture by centrifugation of herparinized venous blood as described (*6*). The leukocyte cultures contained 20 percent autologous plasma, penicillin, and streptomycin, and were incubated in microtiter plates containing 0.2 ml of culture at 37°C in a 5 percent CO_2-humidified atmosphere (*7*). The cultures were assayed for [^{3}H]thymidine incorporation in quadruplicate on the fifth day with a multiautomated sample harvester (Microbiological Associates), and the results are expressed as counts per minute per culture ± the standard error.

Sperm from a donor (J.C., experiment 1, Table 1) was incubated with autologous leukocytes as well as allogeneic leukocytes (D.P., experiment 1). The results are expressed as the uptake of [^{3}H]thymidine in leukocyte cultures with sperm, compared to uptake of [^{3}H]thymidine of identical leukocytes cultured without sperm (leukocytes alone). In some experiments (experiments 3, 9, and 10) more than one allogeneic response was compared to a single autologous result. With fresh suspensions of sperm cells (within 2 hours of ejaculation), high degrees of lymphocyte transformation and DNA synthesis were observed when sperm were added to allogeneic leukocyte cultures, and no significant DNA synthesis was observed when sperm were added to autologous leukocyte cultures (Table 1). There was considerable variation in the degree of stimulation of allogeneic leukocytes with stimulation ratios ranging from 3.2 to 252.

The lymphocyte transformation induced by sperm was dependent on the dose, with maximal stimulation occurring between 1×10^6 and 2×10^6 sperm per milliliter of culture (Fig. 1). Seminal plasma was inhibitory to both allogeneic and autologous leukocytes stimulated with tetanus toxoid