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Pineal gland in rats with 7,12-dimethylbenz (α) anthracene-induced mammary tumors subjected to manipulations known as enhancers of pineal actions

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Summary. The ultrastructure of pinealocytes was studied in rats with 7,12-dimethylbenz (a) anthracene-induced mammary tumors which were subjected to experimental manipulations known as enhancers of pineal actions (anosmia, underfeeding or cold exposure). In these animals we found: (I) - more nuclei with deep nuclear invaginations; (II) - a large number of cytoplasmic organelles, including lipid droplets, myeloid bodies, synaptic ribbons and lysosomes; (III) - numerous degenerative changes. In general, we found an increase in structural features related to pineal photoneuroendocrine activity. Our results indicate that pineal-dependent inhibition of neoplastic growth induced by these experimental manipulations, previously reported, can be mediated through an increase in pineal metabolic activity.

Key words: Pineal gland, Anosmia, Underfeeding, Cold exposure, DMBA-induced tumor

Introduction

Numerous studies have suggested that there is a relationship between the pineal gland and the presence of malignancy. The experiments have generally been based on manipulations such as exposure of animals to different photoperiods (Aubert et al., 1980; Shah et al., 1984), pinealectomy (Aubert et al., 1980; Tamarkin et al., 1981; Shah et al., 1984) or administration of either pineal extracts or melatonin (Aubert et al., 1980; Tamarkin et al., 1981; Blask, 1984; Shah et al., 1984), in rats with 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumors. In general, the pineal gland appears to exert an inhibitory effect on tumor growth. Recent studies on female rats subjected to pineal-enhancing manipulations demonstrate that an increased level of pineal action strongly inhibits both the genesis and the development of mammary neoplasms which have previously been induced (Blask, 1984; Blask et al., 1986; Sánchez-Barceló et al.,, 1988). Morphological and biochemical changes in the pineal gland have been correlated with neoplastic processes in animals and humans (Rodin and Overall, 1967; Hadju et al., 1972; Lapin, 1976; Kerenyi, 1979; Tapp, 1980a,b). To verify whether the influence of the enhanced pineal actions on tumor genesis and growth involves histological alterations in the pineal gland, we studied the morphological appearance of the pinealocytes of rats subjected to experimental manipulations known as enhancers of pineal actions: anosmia (Reiter et al., 1969; Mediavilla et al., 1985; Sánchez-Barceló et al., 1985), underfeeding (Sorrentino et al., 1971), chronic cold exposure (Sánchez-Barceló et al., 1986), or receiving DMBA as mammary carcinogenic agent.

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Materials and methods

In a first experiment, 28-day-old female Sprague-Dawley rats, born in our vivarium, were subjected to some of the following manipulations: blinded + olfactory bulbectomized (BA), blinded + underfed (BU), blinded + exposed to cold (BC), blinded alone (B) or left intact (O). Four weeks later (at 55 days of age) these animals received a single intragastrical dose of DMBA (20 mg in 1 ml of sesame oil). Throughout the experiment, all animals were exposed to a 12/12 light/dark photoperiod and fed «ad libitum» with the exception of rats of the BU group which received daily 30% less food than that consumed by controls. Access to water was free in all cases. Animals of BC group were placed in a room at 10° C; the environmental temperature for the remaining groups was 22°C. All surgical procedures were carried out under i.p.-administered tribromoethanol anaesthesia (25 mg/100 g body weight). Olfactory bulbectomy was performed by aspirating olfactory bulbs

through a 5 mm hole 1 mm posterior to the nasofrontal suture on the midline. Blinding was done by bilateral optical enucleation. At 20 weeks of age animals were sacrificed. In a second experiment, rats bearing tumors typified as adenocarcinomas of similar size were subjected to the experimental manipulations mentioned above. The development of tumors was controlled in this experiment until 9 weeks later. In both experiments, at the time tumor-bearing rats were killed, rats of the same sex, age and weight, that had received sesame oil only (controls), were killed along with the tumor-bearing animals. All rats were sacrificed by decapitation, between 3 and 5 h. after lights on. The pineal gland was fixed in 1% paraformaldehyde at pH 7.4, postfixed in 1% osmium tetroxide, stained in 2% uranyl acetate, dehydrated and embedded in araldite. Sections were cut with an ultramicrotome, stained with lead citrate-Reynold's technique (1963), and examined under a Zeiss EM 109 electron microscope.

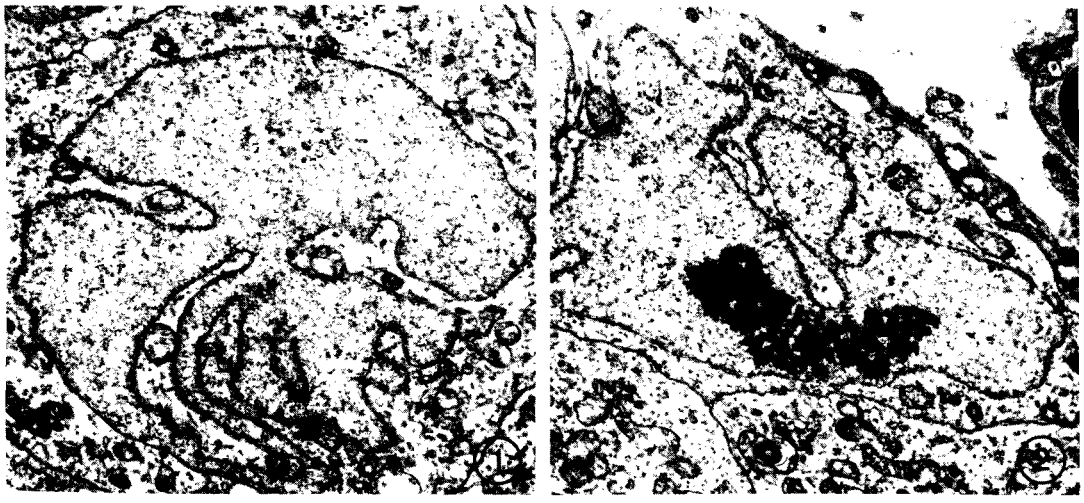
Results

In both experiments, pinealocytes of rats with tumoral inhibition which were subjected to pineal-enhancing manipulations (BA, BC and BU groups) showed greater nuclear size, with deep nuclear invaginations (Figs. 1, 2), than those from O and control animals. The pineal lipid accumulation (Figs. 3, 4) was also greater in these groups than in the others. The control animals showed lower lipid content than the rats which were only blinded or those in the O group (DMBA alone). The cytoplasm of pinealocytes of BA, BU and BC rats showed abundant myeloid bodies (Fig. 5), normally localized in the periphery of the cytoplasm, although some were found in the perinuclear area. Frequently we also observed a large number of cytoplasmic organelles such as synaptic ribbons (Fig. 6) and a large number of lysosomes (Figs. 7, 8). The incidence of these organelles was lower in B animals and they only appeared occasionally in O and control groups. In addition, numerous degenerative changes, such as cyst formations (Fig. 9) and nuclear pyknosis (Fig. 10), were observed in the pinealocytes of the BU, BA and BC rats. These structures rarely appeared in the remaining groups.

Discussion

The morphological features that we found in blind bulbectomized, blind underfed and blind cold-exposed animals, were similar to those described by other authors under various physiological and experimental conditions, which have been regarded as morphological correlates of secretory products or as storage sites for pineal hormones (Vollrath, 1981; Pevet, 1983). The lipid content of the pineal gland, which is known to vary with the environmental lighting and age, has been used as a parameter of activity of the gland (Quay, 1961; González and Blázquez, 1975; Tapp, 1980b). The increase of the pineal lipid content in animals with tumors, with respect to tumor-free animals, has been described previously in rats with DMBA-induced fibrosarcomas (Tapp, 1980b), suggesting that pineal metabolic activity is augmented in the presence of a neoplastic growth. The larger increase in BA, BU and BC rats as compared with the O group, seems to indicate a greater pineal secretory activity in these animals induced by the experimental manipulations.

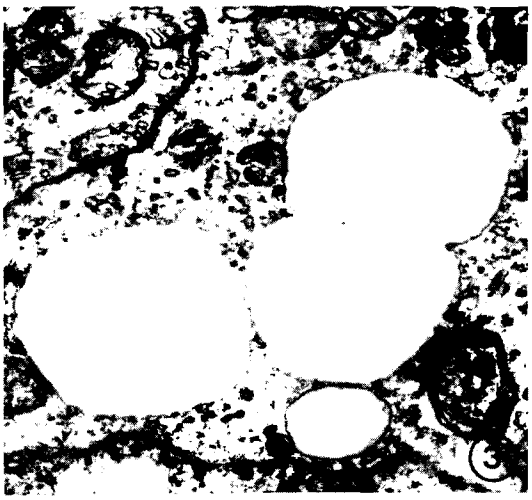
The other findings in blind bulbectomized, blind underfed and blind cold-exposed rats, such as greater nuclear size and increase in the number of myeloid bodies, synaptic ribbons and lysosomes, have been considered in rats blinded or exposed to continual darkness as results of increased pineal function (Vollrath, 1981; McNulty, 1986). The large numbers of these structures observed in experimental models with pineal-dependent gonadal impairment strongly supports the hypothesis of a relationship between these structures and the photoneuroendocrine activity of the pineal gland (Cos et al., 1987). The cyst formations that we found were similar to those described by other authors in patients dying of cancer (Hadju et al., 1972; Tapp, 1980a). Of course, the incidence of these formations is known to vary with age, but in studies of structural alterations due to aging no cyst formations were observed in animals of this age (Johnson, 1980), nor were they observed in our control animals. Although there are no studies of the effect of DMBA on the pineal ultrastructure, we

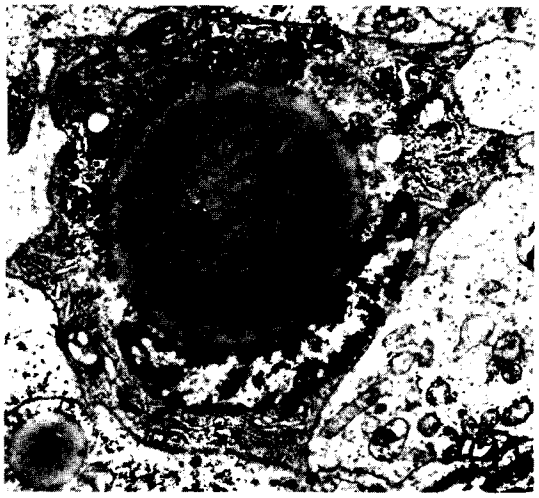


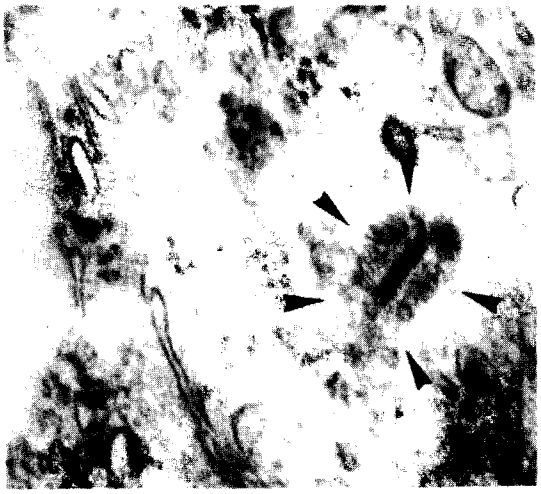
Figs. 1-2. Electron micrograph from blind bulbectomized (Fig. 1) and blind cold-exposed (Fig. 2) female rats. Note the irregular shape of the nuclei and the deep indentations. × 9,000

considered that the increase in the number of these structures was not a consequence of direct damage at pineal level induced by the carcinogenic agent, since they did not appear with the same frequency in rats only treated with DMBA. Relkin (1976) suggested that these degenerative changes were the end results in glands which have been chronically hyperactive in secreting substances which are capable to some extent of controlling neoplasia.

Based on the above data, we suggest that the animals blinded and olfactory bulbectomized, blinded and underfed, and blinded and exposed to cold, show



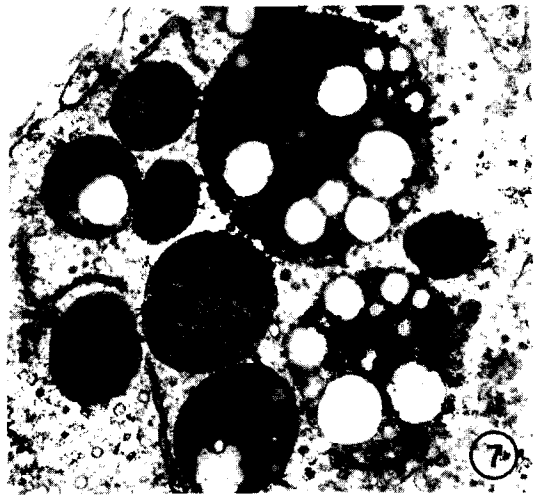


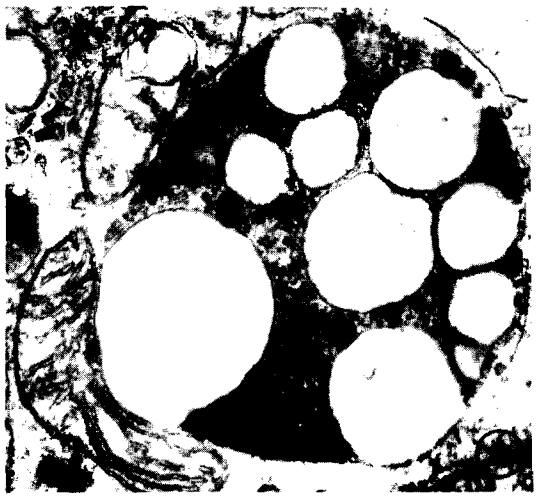


Figs. 3-4. Observe the large number and size of lipid droplets in pinealocytes of rats subjected to manipulations known as enhancers of pineal actions (BU and BC rats). Figure 3 × 25,000 and Figure 4 × 10,000

Fig 5. High magnification electron micrograph showing the association of a myeloid body with a lipid droplet in a light pinealocyte from a blind underfed rat. × 45,000

Fig. 6. Electron micrograph showing synaptic ribbons in the cytoplasm of the pinealocytes of blind cold-exposed rats. × 85,000





Figs. 7-8. Cytoplasm of pinealocytes of rats subjected to pineal-enhancing actions showing abundant lysosomes (BA and BU rats). Figure 7 × 20,000 and Figure 8 × 45,000

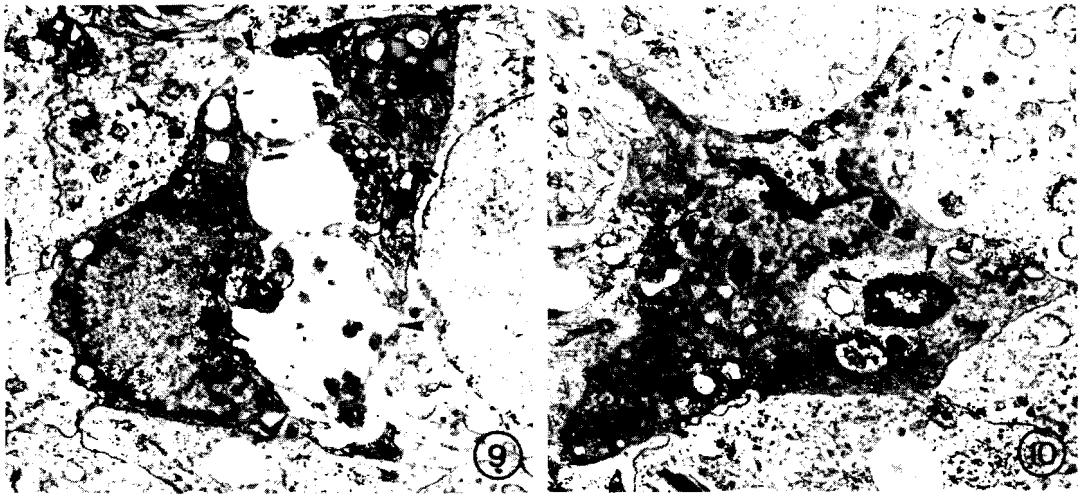


Fig. 9. Electron micrograph showing cyst formations found in pinealocytes of BU, BA and BC rats. × 5,500

Fig. 10. Pinealocyte with nuclear pyknosis from a blind underfed rat. × 7,000

changes in the morphology of the pineal gland that are functionally linked to the photoneuroendocrine activity of the gland. The presence of these structures in animals with pineal-dependent inhibition of neoplastic growth induced by the experimental manipulations mentioned above (Sánchez-Barceló et al. 1988), strongly supports the hypothesis that this inhibition is mediated through an increase in pineal metabolic activity. This provides indirect evidence that the pineal gland itself may be involved in the control of malignancy.

References

Aubert Ch., Janiaud P. and Lecalvez J. (1980). Effect of pinealectomy and melatonin on mammary tumor growth in Sprague-Dawley rats under different conditions of lighting. J. Neural Transm. 47, 121-130.

Blask D.E. (1984). The pineal: an oncostatic gland?. In: The Pineal Gland. Reiter R.J. (ed). Raven Press. New York. pp 253-284.

Blask D.E., Hill S.M., Orstead K.M. and Massa J.S. (1986). Inhibitory effects of the pineal hormone melatonin and underfeeding during the promotional phase of 7,12-dimethylbenzanthracene - (DMBA) - induced mammary tumorigenesis. J. Neural Transm. 67, 125-138.

Cos S., Bardasano J.L., Mediavilla M.D. and Sánchez-Barceló E.J. (1987). Myeloid bodies associated with lipid droplets in pinealocytes of blind, blind-bulbectomized, blind-underfed or blind cold-exposed rats. Neurosci. Lett. 74, 119-122.

González G. and Blázquez E. (1975). Ultrastructural evidence of a secretory process in the rat pineal gland. Experientia 31, 969-971.

Hajdu S.I., Porro R.S., Lieberman P.H. and Foote F.W. (1972). Degeneration of the pineal gland of patients with cancer. Cancer 29, 706-709.

Johnson J.E. (1980). Fine structural alterations in the aging rat pineal gland. Exp. Aging Res. 6, 189-211.

Kerenyi N.A. (1979). Tumors and the pineal gland. In: Influences of hormones in tumor development. vol. I. Kellen J.A. and Hilf R. (eds). CRC Press. Boca Raton. pp 155-165.

Lapin V. (1976). Pineal Gland and Malignancy. Oster. Z. Onkol. 3, 51-60.

McNulty J.A. (1986). Phylogeny and functional implications of synaptic ribbons in the pineal gland. IRCS. Med. Sci. 14, 855-858.

Mediavillla M.D., Sánchez-Barceló E.J., Sánchez-Criado J.E., Cos S. and Cortines M.D.G. (1985). Antigonadal actions of olfactory and light deprivation. II. Effects of pinealectomy or melatonin injections in olfactory bulb deafferented or bulbectomized male rats. J. Pineal Res. 2, 191-200.

Pevet P. (1983). Anatomy of the pineal gland of mammals. In: The Pineal Gland. Relkin R. (ed). Elsevier Biomedical. New York. Amsterdam. Oxford. pp 1-75.

Quay W.B. (1961). Reduction of mammalian pineal weight and lipid during continuous light. Gen. Comp. Endocrinol. 1, 211-217.

Reiter R.J., Klein D.C. and Donofrio R.J. (1969). Preliminary observations on the reproductive effects of the pineal gland in blinded anosmic male rats. J. Reprod. Fert. 19, 563-565.

Relkin R. (1976). The pineal. Lancaster. Eden Press. England.

Reynolds E.S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17, 208-212.

Rodin A.E. and Overall J. (1967). Statistical relationships of weight of the human pineal to age and malignancy. Cancer 20, 1203-1214.

Sánchez-Barceló E.J., Mediavilla M.D., Sánchez-Criado J.E., Cos S. and Cortines M.D.G. (1985). Antigonadal actions of olfactory and light deprivation. I. Effects of blindness combined with olfactory bulb deafferentiation, transection of vomeronasal nerves, or bulbectomy. J. Pineal Res. 2, 177-190.

Sánchez-Barceló E.J., Cos S. and Mediavilla, M.D. (1986). Cold exposure as a potentiating factor of pineal actions in nonhibernating mammals. Cryobiology 23, 72-80.

Sánchez-Barceló E.J., Cos S. and Mediavilla M.D. (1988). Influence of pineal gland function on the initiation and growth of hormone-dependent breast tumors. Possible mechanisms. In: The Pineal Gland and Cancer. Gupta D., Attanasio A. and Reiter R.J. (eds). Brain Research Promotion. pp 221-232.

Shah P.N., Mhatre M.C. and Kothari L.S. (1984). Effect of melatonin on mammary carcinogenesis in intact and

pinealectomized rats in varying photoperiods. Cancer Res. 44, 3403-3407.

Sorrentino S., Reiter R.J. and Schalch D.S. (1971). Interactions of the pineal gland, blinding and underfeeding on reproductive organ size and radioimmunoassayable growth hormone. Neuroendocrinology 7, 105-115.

Tamarkin L., Cohen M., Roselle D., Reichert Ch., Lippman M. and Chabner B. (1981). Melatonin inhibition and pinealectomy enhancement of 7,12-dimethylbenz(a)anthracene-induced mammary tumors in the rat. Cancer Res. 41, 4432-4436.

Tapp E. (1980a). The human pineal gland in malignancy. J. Neural Transm. 48, 119-129.

Tapp E. (1980b). Pineal gland in rats suffering from malignancy. J. Neural Transm. 48, 131-135.

Vollrath L. (1981). The pineal organ. In: Handbuch der Mikroskopischen Anatomie des Menschen. volumme VI/7. Oksche A. and Vollrath L. (eds). Springer-Verlag. Berlin Heidelberg New York.

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