Morphodynamic response of the rat light pinealocytes to an injection act. Implication of ß-adrenoreceptors

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Summary. The implication of ß-adrenoreceptor-mediated noradrenalin action on the reactive morphodynamic response of light pinealocytes (LP) to an injection act, conceived as a short-lasting stress attack, is reported. An injection act, realized by saline-injection, was manifested in the occurrence of clustered «dusk» and «bright» cells - the representative forms of functionally activated LP. The time-related incidence of these cells entities - the appearance of «dusk» and «bright» cells at 5 min, transitory domination of «bright» cells and the nadir of «dusk» cells at 20 min, sporadic recognition of «bright» cells at 45 min and the lack of «dusk» cells at 45 min and the absence of both cell forms at 180 min - displayed that LP-reactive response promptly appeared and rapidly ceased. The injection of ß-adrenoreceptor antagonist, propranolol, did not considerably alter the pattern of LP-reactive response proper for saline-injected rats at 5 min. The constant presence of «dusk» and «bright» cells, structurally changed in all the following time periods under investigation, showed that this drug disordered the course of LP-reactive response to an injection act. On the contrary, LP of resting state was not found to be affected. Estimating functionally, the present results indicate that ß-adrenoreceptor-mediated noradrenalin action is required to promote, maintain and accomplish the LP-reactive response to a short-lasting stress inducement.

Key words: Light pinealocytes, ß-adrenoreceptors, Noradrenalin, Saline injection, Short-stress inducement

Introduction

The pineal gland is joined to the system of organs engaged in the coordination and integration of a reactive response of the organism to stress (Miline, 1980; Reiter, 1988). Several studies have shown the ability of LP, the main if not the unique endocrine cell of the pineal gland parenchyma, to promptly meet stress inducement (Miline et al., 1989; Segie et al., 1985). Following the generally accepted concept that stress is initially determined by an evoked action of sympathicus and that this multi-component system is crucially implied in the regulation of LP physiology (Reiter, 1984; Govitrapong et al., 1989), its participation in the stress-reactive animation of LP could not be neglected. When bringing noradrenalin into focus, such an approach was consonant with some previous studies concerning LP indolaminergic activity (Joshi et al., 1986; Vollrath and Welker, 1988). However, its role in stress-reactive LP morphodynamic animation has been studied very little (Matsushima and Morisawa, 1980). Therefore, the present study was designed to expand the knowledge of the implication of this regulative variable, not only in the promotion, but also in the course of a reactive morphodynamic response of LP to an injection act, conceived as a short-lasting stress inducement.

It is well known that noradrenalin acts on LP through both α1- and ß-adrenoreceptors. The latter are essentially needed, while their mediating effects are amplified by the former (Ho and Chick, 1990). Accordingly, it was thought appropriate to examine the effect of the antagonism of ß-adrenoreceptors on the promotion and the course of LP stress-reactive response. The idea was realised on the grounds of the previous results demonstrating that the injected drug affected already functionally impelled LP (Milin, 1988) due to an instantaneous LP reactivity to the injection act itself and the latency responding to its kinetic parameters.

Materials and methods

3-month-old male Wistar rats, weighing 150-180 g, kept under controlled laboratory living conditions of light/dark regime of 14/10 hrs, were used in the experiment. Food and water were given ad libitum.

– Control animals. The rats were decapitated after removal from the cage. The group consisted of 4 rats.

– Saline-injected animals. The rat was taken out from the cage, weighed, intraperitoneally injected with saline (0.83%, 1 ml per 100 g of body weight), immediately put back into the cage and left undisturbed. The whole manipulative procedure lasted 15-20 sec. In each post-injection time period, scheduled at 5, 20, 45 and 180 min, 4 rats were sacrificed.

– Propranolol-injected animals. As previously, the rat was removed from the cage, weighed, intraperitoneally injected with propranolol, selected ß-adrenoreceptor antagonist (10 mg/1 ml per 100 g of body weight), placed back into the cage and left peacefully. In each post-injection time period scheduled at 5, 20, 45 and 180 min, 4 rats were sacrificed.

The post-injection time interval was determined in accordance with the drug plasma half-life (Goodman and Gilman, 1975).

The experiment was done in the morning hours.

Electron microscopy

Immediately after decapitation, the skull was opened, the pineal gland dissected and prefixed in 2.5% glutaraldehyde/0.1 M cacodylate buffer pH 7.4 for 2 hours, fixed in 2% osmium tetroxide/0.1 M cacodylate buffer pH 7.4 for another 2 hours, and then dehydrated and embedded in Epon 812. Successively taken sections were collected and routinely contrasted with uranyl acetate and lead nitrate. The examination was performed in a Zeis 109 electron microscope.

The procedure of immersion, instead of perfusion, was employed to prevent an interaction of injection stress with surgery stress.

Morphometric analysis

The range occurrence of functionally animated LP -their «dusk» and «bright» cell forms, as well as LP of resting state, were quantified directly on the screen at the magnification of × 4,400 in randomly selected parts of the gland parenchyma lying, at least, over 10 grid holes. Since each grid hole measures 75 μm², and more than 10 grids were examined, the reference area of each pineal gland parenchyma was larger than 7,500 μm². The cells possessing nucleus, but not cell processes, were scored. The results were expressed as the percentage of the total number of the counted LP. The data were average (mean ± se), statistically estimated using one-way analysis of variance (ANOVA) and the Mann-Whitney U-test.

Results

Electron microscopy

Control animals

Since the morphodynamic properties of LP in the state of resting are already well known (Wolfe, 1965; Arstila, 1967), there is no need to give them any particular consideration.

Saline-injected animals

- 5 min. The basic impression of the cellular organization of the gland parenchyma came from the presence of different forms of functionally animated LP distributed alone or amassed in clusters. Cells marked by an electron dense cytoplasmic matrix, possessing flattened or slightly dilated GER tubuli, unequally disseminated polysomes, Golgi apparata of rather active feature and mitochondria of an orthodox or energized configuration were identified as «dusk» cells. The extrusion of structures traditionally termed lipid droplets (Fig. 1), together with accumulated clear vesicles within the apical portions of the cell processes and the sporadic occurrence of omega figures, exhibited their engaged secretory activity. Moreover, the presence of the cells deprived of lipid droplets, though populated by many polysomes and usually active Golgi apparata, indicated that «dusk» cells might also represent post-secretory LP. Cells with swollen mitochondria were sporadically noticed (Fig. 2). Parenthetically, respecting the current nomenclature, «dusk» cells do not correspond to dark pinealocytes. LP having a lucent cytoplasmic matrix, plenty of polysomes, fasciculated or broadened GER tubuli (Fig. 3) and multiloculated Golgi apparata of an active feature (Fig. 5), were recognized as LP of an intensified elaborative activity. They were designated as «bright» cells. The leaning of enlarged membranous profiles on the cell membrane and wide «openings» into intercellular space (Fig. 4) showed their involvement in the supply of the pineal gland causing secretory response as well.

The remainder of LP displayed the usual structural organization for their resting state.

- 20 min. «Dusk» cells were less numerous. In contrast, «bright» cells were more frequent. The impression of their increasing elaborative activity was given by crater-like aggregated polysomes around the newly appearing lipid droplets (Figs. 6, 7).

- 45 min. The absence of «dusk» cells and a reduction in «bright» cells marked this time period.

- 180 min. Structural properties of LP did not essentially differ from the cells in control animals.

Propranolol-injected animals

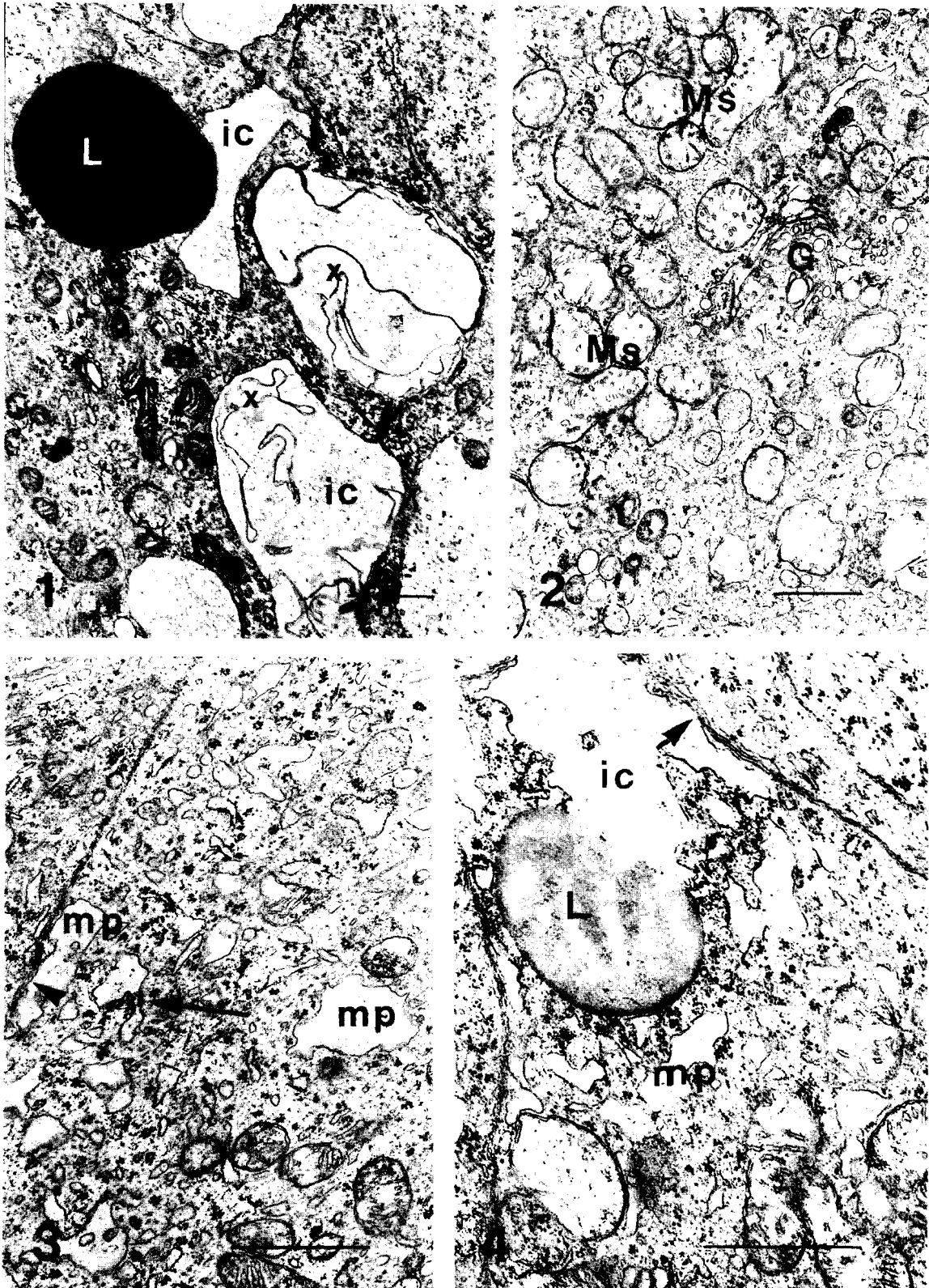- 5 min. The clusters of activated LP (Fig. 8) demonstrated the propranolol injection did not

Fig. 1. Saline-injected rat. 5 min. Secretory engaged «dusk» cell. Extrusion of lipid droplet (L) into pearl-like intercellular space (ic). Convoluted membranous remnant (x) of a postdischarged lipid droplet. Scale bar = 1 μm.

Fig. 2. Saline-injected rat. 5 min. «Dusk» cell populated by swollen mitochondria (Ms). Golgi apparatus with active features (G). Scale bar = 1 μm.

Figs. 3 and 4. Saline-injected rat. 5 min. «Bright» cells. Dilated GER tubule (big arrow) giving rise to membranous profiles (mp). The leaning of such a profile on cell membrane (arrowhead) and fusion of both membrane (small arrow) leads to a wide «opening» of membranous profile into intercellular space (ic). Lipid droplet (L). Scale bar = 1 μm.
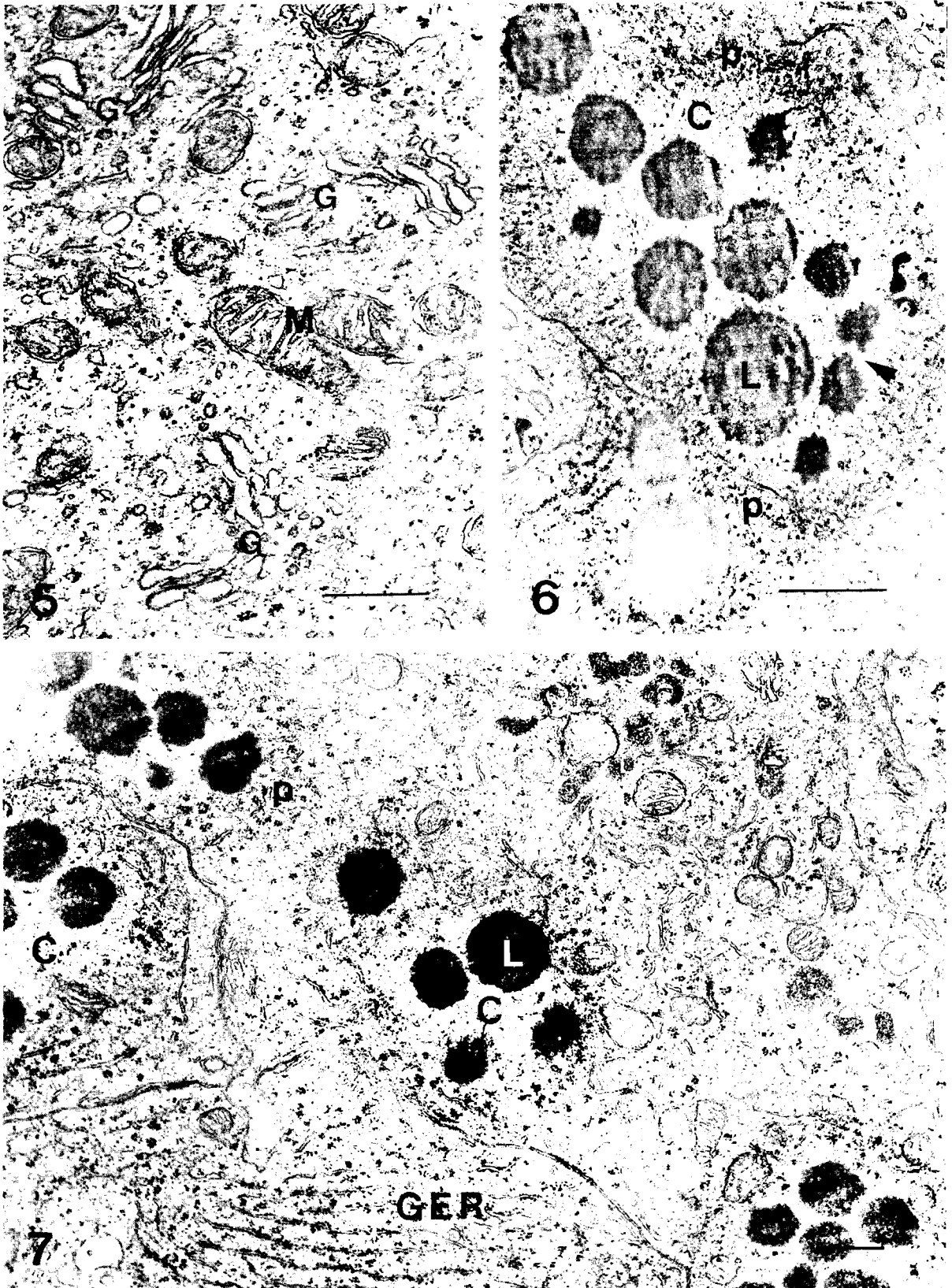
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Fig. 5. Saline-injected rat. 5 min. «Bright» cell. Multiloculated active Golgi apparata (G). Energized mitochondria (M). Scale bar = 5 µm.

Figs. 6 and 7. Saline-injected rat. 20 min. «Bright» cells. Crater-like aggregated polysomes (p), patches of condensed material (arrowhead) growing into lipid droplets (L). Fasciculated GER tubuli (GER). Scale bar = 1 µm.
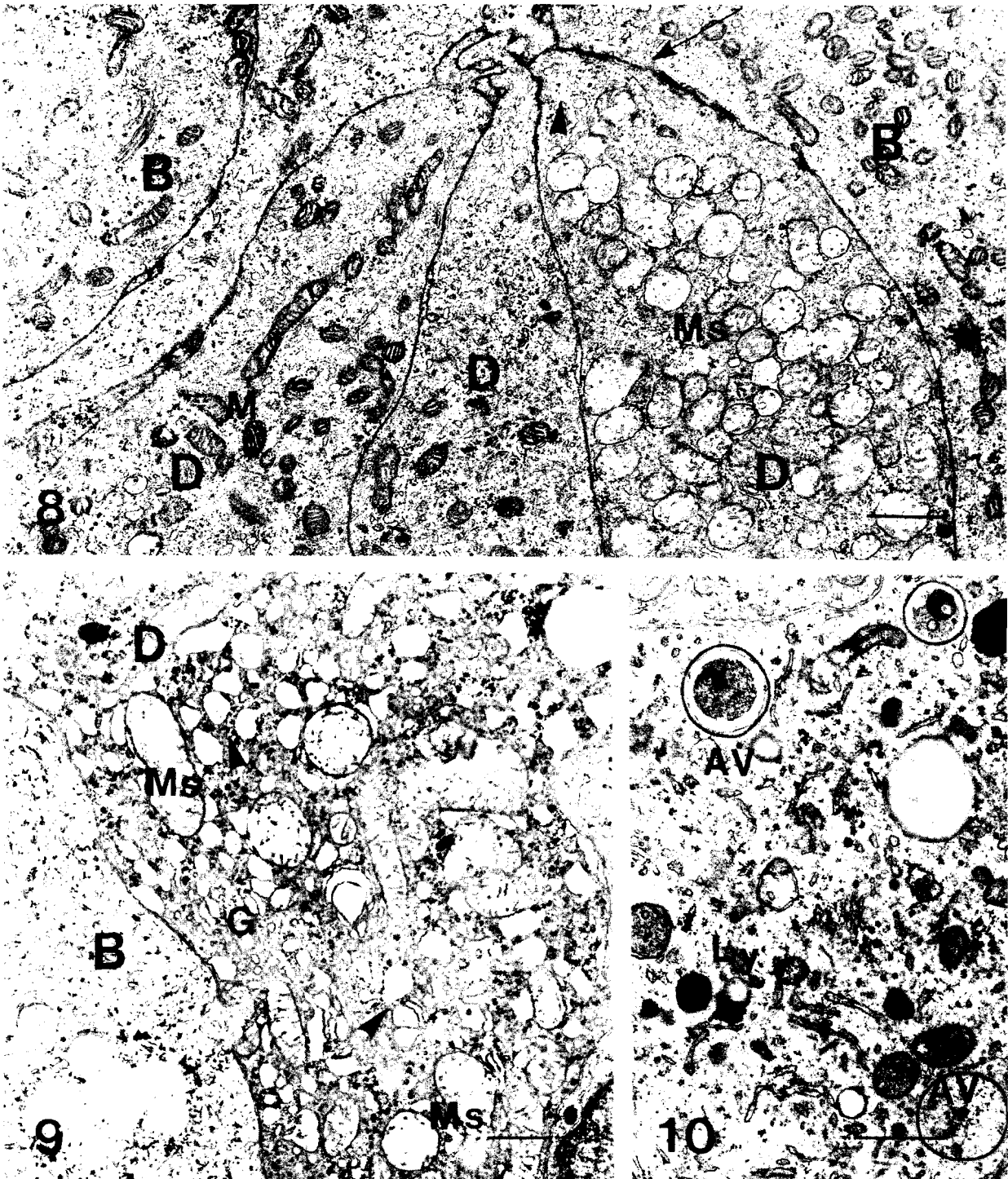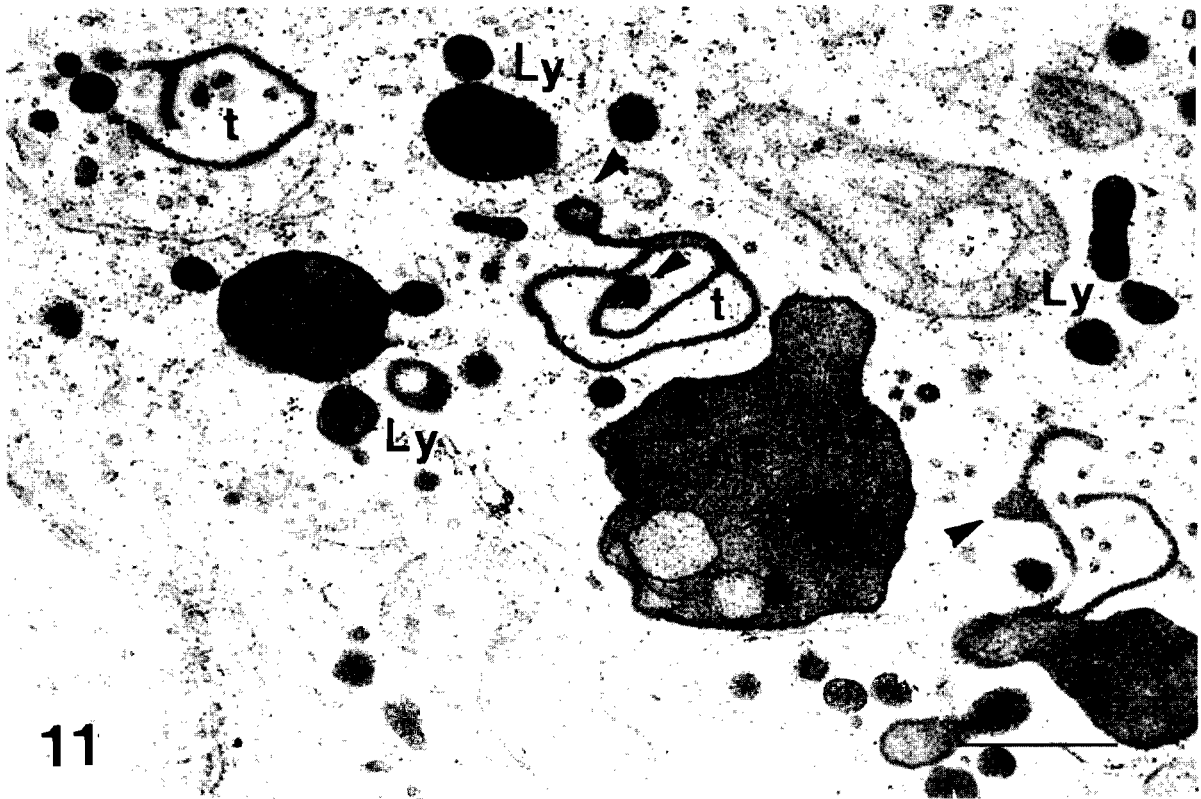
Fig. 8. Propranolol-injected rat. 5 min. A cluster of pseudofollicularly-joined processes of «dusk» (D) and «bright» (B) cells. Clear vesicles (arrowhead), energized (M) and swollen mitochondria (Ms). Gap junctions (arrow). Scale bar = 1 μm.
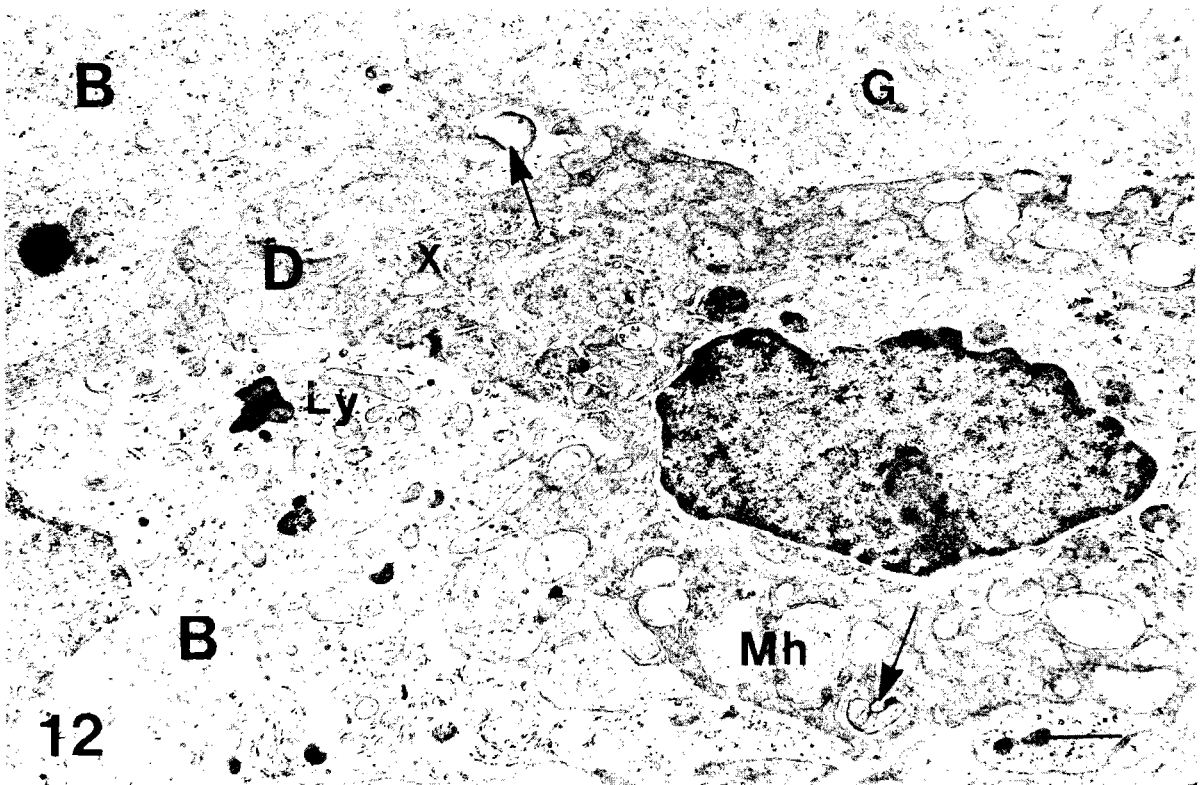
Fig. 9. Propranolol-injected rat. 20 min. «Dusk» cell. Note highly condensed cytoplasm (compare with «bright» cell - B). Cystically-dilated endoplasmic reticulum (arrowhead) and swollen mitochondria (Ms). Golgi apparatus (G). Scale bar = 1 μm.

Fig. 10. Propranolol-injected rat. 20 min. «Bright» cell. Lysosomes (Ly) and autophagic vacuole (AV). Scale bar = 1 μm.

Fig. 11. Propranolol-injected rat. 45 min. «Bright» cell having conspicuously undergone hydrolytic changes - twisted tubuli filled with highly condensed material (t) giving rise to small lysosome (arrowhead). Amassed lysosomes (Ly). Autophagosome (AF). Scale bar = 1 μm.

Fig. 12. Propranolol-injected rat. 180 min. «Dusk» and «bright» cells. Both cell types are poor in polysomes. «Bright» cell (B) possesses focally-accumulated GER tubuli (x), hydropic mitochondria filled with fine-grained content (Mh) and subjected to myelin-like degeneration (arrow). «Bright» cells (B) are marked by dishevelled GER tubuli, Golgi apparatus (G) and lysosomes (Ly). Scale bar = 1 μm.

Fig. 13. Propranolol-injected rat. 180 min. «Dusk» cell with apparently enlarged perinuclear cisternae and GER tubuli (x). Golgi apparatus (G). Swollen and degenerated mitochondria (arrow). Scale bar = 1 μm.

essentially alter the pattern of an initial reactive response of LP multitude to an injection act.

– 20 min. There was a regional presence of «dusk» cells, polymorphous in shape. Most of them possessed nuclei with marginated heterochromatin, plenty of polysomes, active Golgi apparata, but swollen mitochondria and cystically dilated endoplasmic reticulum (Fig. 9). «Bright» cells were abundant in polysomes and dishevelled GER tubuli. Solitary displaced mitochondria were of an energized nature. Lipid droplets were rare. However, lysosomes and autophagic vacuoles were found (Fig. 10).

– 45 min. In contrast to saline-injected rats, «dusk» and «bright» cells were observed in this time period. Regarding the former, there were cell samples with a deeply enfolded nucleus of marginally clumped heterochromatin, enlarged perinuclear cisternae and apparently dilated endoplasmic reticulum. Although Golgi apparata were still of an active feature, the absence of lysosomes indicated that the hydrolytic enzyme system was not activated. Mitochondria exhibited massive swelling and distortion of cristae. The common characteristics of «bright» cells were reduced polysomes, dishevelled GER tubuli and the disappearance of polysomes from endoplasmic tubuli. Some mitochondria underwent swelling. Lysosomes and autophagic vacuoles were observed in a larger number of the cells. There were also cell samples occupied with an intensified hydrolytic process

- twisted GER tubuli filled with electron dense material (tubular lysosomes - Krstic, 1988), giving rise to small lysosomes and autophagosomes (Fig. 11).

– 180 min. «Dusk» and «bright» cells were still present. The reduction in polysomes, enlargement of perinuclear cisternae and GER tubuli, hydropic mitochondria and altered myelin-like ones illustrated a progress in the structural regression of «dusk» cells (Fig. 12). The cells with apparently enlarged perinuclear cisternae and GER tubuli were present as well (Fig. 13). «Bright» cells were marked by scarce polysomes, dishevelled GER tubuli and scattered lysosomes (Fig. 12).

Collectively considered, LP of resting state did not display marked structural changes after propranolol injection during the whole time period under investigation.
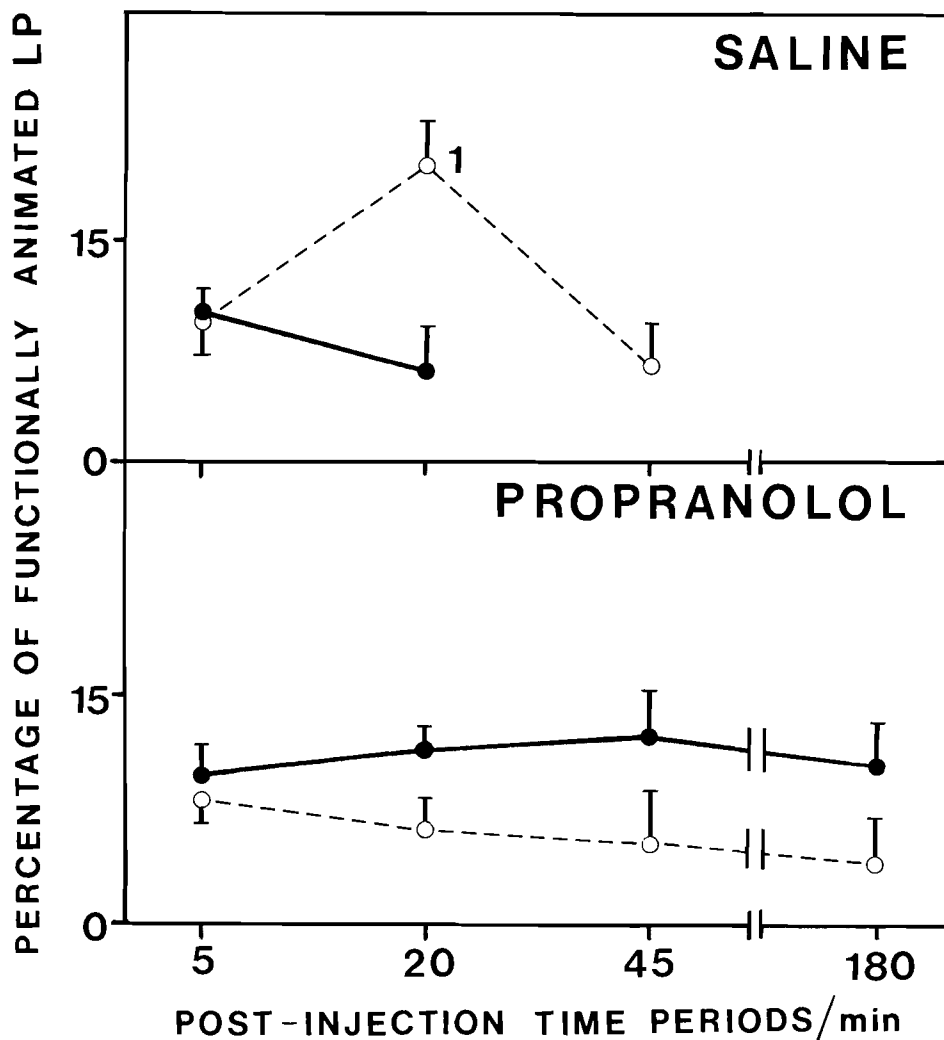
Morphometry

The cell scoring showed an almost equivalent occurrence of «dusk» and «bright» cells at 5 min after saline-injection. The former cells were in their nadir at 20 min and disappeared in the subsequent time periods. The latter cells were considerably numerous at 20 min, apparently reduced at 45 min and missing at 180 min (Graph 1, saline).

The incidence of both cell types in propranolol-injected rats, at 5 min, insignificantly differed with

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Graph 1. Time course incidence of "dusk" cells (solid lines) and "bright" cells (dotted lines) in saline- and propranolol-injected rats. Values are a mean ± SE.
1. Significant difference in "bright" cell appearance in 5 and 45 min, p < 0.05.

PERCENTAGE OF FUNCTIONALLY ANIMATED LP

SALINE

PROPRANOLOL

15

0

15

0

5   20   45   180

POST-INJECTION TIME PERIODS/min

respect to the saline-injected rats. The similar rate in the presence of «dusk» and «bright» cells was found throughout the further time periods scheduled (Graph 1, propranolol).

Discussion

The reactive response to saline-injection was recognized by the occurrence of clustered «dusk» and «bright» cells - the representative cell forms of functionally animated LP. In physiological terms, «dusk» cells were thought to be strikingly-secretory activated LP, or their post-secretory entities. «Bright» cells were qualified as LP of highly stimulated elaborative activity (Milin et al., 1984). Considering the time related incidence of both cell types - the appearance of both cell forms at 5 min, transitory domination of «bright» cells and the nadir of «dusk» cells at 20 min, sporadic recognition of «bright» cells and the absence of «dusk» cells at 45 min, as well as

the lack of the latter at 180 min - it comes out that LP-reactive response to an injection act promptly appears and rapidly ceases. It, therefore, gives an impression that LP of resting state possess very dynamic response-recovery capabilities to an injection act. However, the reason why a part, but not the whole LP multitude, responded to stress inducement requires further consideration.

The incidence of «dusk» and «bright» cells 5 min after propranolol injection was not considerably different with respect to the same time period in saline-injected rats. The permanence of both cell forms in the following time period pointed out that propranolol interrupted the course of reactive response of functionally animated LP. However, with respect to the divergence in their structural changes, it was indicative that drug effect might depend on the functional state of the activated LP in which ß-adrenoreceptors were blocked. When focusing on «dusk» cells, it seemed that the antagonism of

ß-adrenoreceptors in the initial moment of activation of LP caused an arrest of their morphodynamic turnover. In the case of «bright» cells, it appeared that the blocking of ß-adrenoreceptors in the period of mounted LP elaborative activity curtailed their already stimulated synthetic processes. If LP is a homogeneous population with respect to the ß-adrenoreceptors, the observation that functionally animated LP were only affected, excluding those of resting state, evidenced that propranolol exerted its profound effect only in the case when the target cell was under an increasing sympathetic influence (Goodman and Gilman, 1975; Stehle et al., 1989).

In attempting to interprete the divergence in the structural changes between «dusk» and «bright» cells - mitochondrial swellings and an enlargement of the endoplasmic reticulum in the former, versus a decrease in polysomes, GER tubuli and appearance of lysosomes in the latter - it is worth remembering the events that occurred in response to the activation/antagonism of ß-adrenoreceptors. It is well known that their induction leads to the hyperpolarisation of LP cell membrane, an elevated influx of Ca^{++} and an augmented production of cyclic nucleotides (Freschi and Parfitt, 1986; Ho and Chik, 1990). Since their antagonism stabilized the turnover of LP cell membrane by preventing the reduction of its hyperpolarization (Emrich and Zerssen, 1983; Foehring et al., 1989), the disorder of the transmembranous Ca^{++} current or the impairment of the production of cyclic nucleotides (Morgan et al., 1989) could be expected. In this sense, the finding that pinealocytes resembling «dusk» cells contained abundant deposits of Ca^{++} (Pizarro et al., 1990) and that an injection of Ca^{++} channel blocker (nifedipine) did not elicit the occurrence of «dusk» cells (in preparation) suggests that the appearance of «dusk» cells is somehow associated with an uncontrolled increase of Ca^{++} influx. The fact that the enlargement of endoplasmic reticulum and the mitochondrial swellings in part of the mechanism involved in lowering the cytoplasmic Ca^{++} level (Trump et al., 1981; Somlyo et al., 1985) favours the present observation of such changed organelles to supplement the outlined assumption. Furthermore, the evidence that an evoked noradrenalin influence is manifested in a rapid increase of cyclic nucleotide production (Sugden, 1989) and progressive LP structural changes (Steinberg et al., 1981; Karasek et al., 1990), points out that structural changes of «bright» cells are probably related to the decline of elevated ß-adrenoreceptor-cyclic nucleotide-mediated processes. Certainly, such an approach should be given separate consideration.

Founded on the principle that pharmacologically-induced changes anticipate the physiological mechanism involved, the permanence of «dusk» cells after propranolol injection indicates that their occurrence in saline-injected rats is associated with an abolished ß-adrenoreceptor-mediated noradrenalin action. If the fluctuation is NAT activity (rate limiting enzyme in melatonin synthesis - Reiter, 1984) is employed as a parameter of noradrenalin action, the depression in the activity of this enzyme (Joshi et al., 1986) might mirror the fact that a fall in an evoked noradrenalin influence happened after saline injection. Thus, it is likely that the «dusk» cell occurrence is really related to a drop in evoked noradrenalin action, feasibly at the moment the injected rat is returned into the cage. Therefore, it comes out that «dusk» cells have to be considered rather as the promptly activated LP whose morphodynamic turnover was transitorily interrupted due to the fluctuation of noradrenalin influence, than the obligatory intermediaries of promptly functionally animated LP.

The question could be posed as to whether «dusk» cells are rather preparative artefacts, at least with respect to the «dark cell-like cell» concept (Ghadially, 1988). However, the dynamics of their occurrence - their absence in control rats, transitory presence in functionally activated pineal gland and their permanence after the antagonism of ß-adrenoreceptors - could hardly support such a possibility.

Collectively viewd, the palette of morphodynamic events that occurred in propranolol-injected rats, indicates that the antagonism of ß-adrenoreceptors disordered the course of reactive response of LP to an injection act. Thus, it might be assumed that an evoked noradrenalin action is required to promote, maintain and accomplish LP stress-reactive response. Although the present results suggest that its involvement is essential for inducing an LP reactive response, the observation that D2-receptor-mediated dopamine action might buffer LP reactive response (Savic et al., 1989), while ACTH facilitated the elaborative activity of functionally activated LP (Milin et al., 1989), indicates that LP stress-reactive response is rather under a multicooperating regulative influence.

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