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Adrenovascular Alterations from Neurogenic Stress Modified by Denervation of the Adrenal Gland in Rat

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The application of a painful stimulus to restrained rats provokes conspicuous adrenovascular alterations (vasodilatation, edema, small hemorrhages). Hypophysectomy does not prevent the adrenovascular response to stress, or the abdominal vagotomy. On the contrary, splanchnicotomy practically abolishes such response. These results show that the vascular alterations, consequence of the action of a neurogenic stress, are not caused by a hypophyso-hormonal response but by a sympathetic one.

Key words: Adrenal, Adrenal lesions, Neurogenic stress, Adrenal denervation.

Adrenal vascular alterations are a common event in humans (12, 14, 17, 23, 34) and in other mammals (4, 15, 38). Sometimes such lesions are provoked by toxins such as those of diphteria and meningococcal infections, (8, 37) and their pathogenesis is well known since TONUTTI's experiments (32, 33), who showed that the diphteria toxin, without the help of ACTH did not cause a necrotic hemorrhage of the adrenals in the guinea pig, and neither did the administration of large doses of ACTH. However, both factors together give rise to a massive adrenal hemorrhage. This toxic etiology of the adrenovascular lesions is infrequent, at least in humans. The great number of such lesions are due to neurogenic stress. Since the rat presents a noticeable adrenovascular reactivity (4, 5) to neurogenic stress, it offers an excellent experimental model to investigate the role of the different neural elements on such vascular lesions. In order to clarify the possible participation of the hypophysis and the autonomic nervous system in the adrenovascular reaction to a neurogenic stress, we have undertaken the following experiments: hypophysectomy, adrenal transplantation, adrenal enuclea-

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tion and neurotomy of the vagus or splanchnicus nerves.

Materials and Methods

Wistar rats of 230-250 g b.w. housed in cages in a room at a constant temperature (21° ± 2 °C), and diurnal illumination, with food and water *ad libitum* were used. The neurogenic stress was provoked by immobilization of the animals and application of pin-pricks on the dorsum for 5 min. The rats were decapitated 90 min after the end of the stress.

Experimental groups. — Control, hypophysectomy, left splanchnicotomy, left vagotomy, bilateral transplantation of adrenals and bilateral enucleation of adrenals. The number of animals in each group was 10, 5 under and 5 free of stress.

Methods. — Hypophysectomy was carried out, under anesthesia with ether, via transauricular route (5, 7, 30). The other surgical interventions were performed under anesthesia with nembutal (30 mg/kg). Splanchnicotomy and vagotomy were done subdiaphragmatically. The rats with transplanted and enucleated adrenals received an adequate survival diet. The recovery times were different depending on the groups. Hypophysectomized rats were decapitated 24 hours after surgery and the animals with transplanted and enucleated adrenals after 60 days, and those with neurotomy after 30 days. At necropsy the infundibulum of the hypophysis was carefully observed and the cases in which a fragment of the adenohypophysis remained were rejected. In the case of rats with neurotomy a fragment of 2 mm was ablated and at necropsy both extremes of the sectioned nerve were examined. There was no continuity between the extremes in any of the rats. With the exception of the glomerulosa and the outmost part of the outer fasciculata, the medulla and cortex were extruded through a small incision of the capsule, in both the transplanted and enucleated adrenals. After decapitation the adrenals were removed and fixed in formalin (10 %), processed for inclusion in paraffin, cut (7 µm) and stained with hematoxylin-eosine and Masson's tricromic method. For the enucleated and transplanted adrenals the Bielschowsky method was used. To objetify the vascular changes in the different experiments, drawings have been made of the capillary bed and hemorrhagic territories, by means of a camera lucida attached to a microscope. The outer and inner border of the adrenal cortex were also outlined. With the aid of a digitizer attached to a computer, the areas of the vascular bed and the cortex were measured. The ratio: vascular area/cortex area will be expressed in percentages.

Results

Control group. — The adrenals of the control animals showed the characteristic structure described for the rat and did not present any vascular alterations (fig. 1). The area of the capillary bed of the cortex was 0.9 % of the cortical area. The stressed rats exhibited very apparent adrenovascular lesions: small hemorrhages, more frequent in the zona reticularis (ZR) and in the deep layer of the zona fasciculata (ZF) (fig. 2), diffuse areas with erythrocytic extravasations and edema. These vascular alterations gave the ZR the appearance of a compressed zone (fig. 3). The area of the hemorrhages occupies 3.1 % and the capillary bed the 5.7 % of the cortex surface.

Hypophysectomy. — The hypophysectomized rats, 24 hours after surgery, appeared perfectly recovered and their adrenals did not show any difference when compared with those of the control animals. The adrenals of the hypophysec-

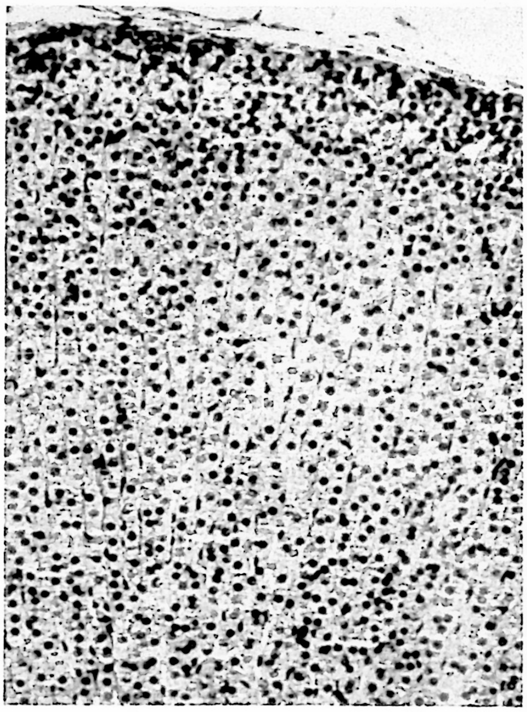

Fig. 1. *Adrenal cortex of an unstressed control rat. (190 ×).*

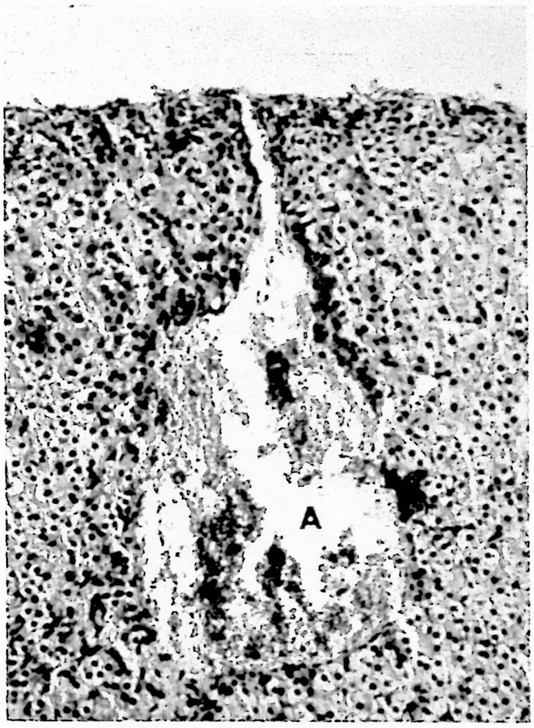

Fig. 2. *Adrenal cortex of a stressed control rat with a hemorrhage in zona fasciculata (A). (190 ×).*

tomized and stressed rats also showed a similar reaction to that observed in the control stressed rats: hemorrhages, edema, and vasodilatation (fig. 4). Area of hemorrhage, 2.9 % and capillary bed, 5.6 %.

Autotransplanted adrenals. — The regeneration of the cortex in the autografted adrenals was complete 60 days after surgery. The outer layer of the ZF occupied more extension than the inner one and the ZR. The adrenal medulla failed to regenerate in all cases and in its place connective tissue appeared. No nerve fibres were observed in the adrenal cortex.

The vascular pattern was similar to that of the control animals and in the parenchyme there were no vascular alterations. The stressed rats did not show adrenovascular lesions, or vasodilatation, i.e., these adrenals did not differt from the non-stressed. Capillary area: 1.1 %.

Enucleated adrenals. — The regeneration of the cortex was complete at the end of 60 days survival, and its structure and vascularization was normal. No vascular alterations were observed in the parenchyme of the gland. On the contrary, the adrenals of the stressed animals showed a vascular reaction similar to the adrenals of the control animals: hemorrhages, edema and vasodilatation. Nerve fibres were present in the regenerated cortex. Hemorrhage area: 2.1 %; capillary bed: 5.4 %.

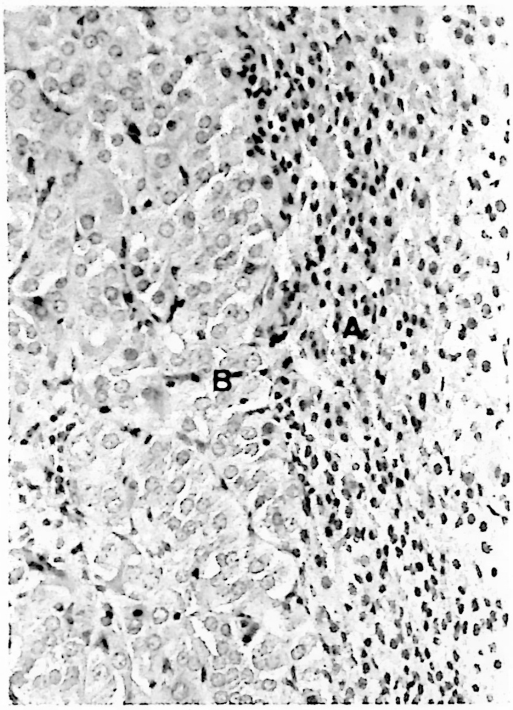

Fig. 3. *Adrenal gland of a stressed rat.* A) Medulla; B) zona reticularis, compressed by the edema of the zona fasciculata interna. (190 ×).

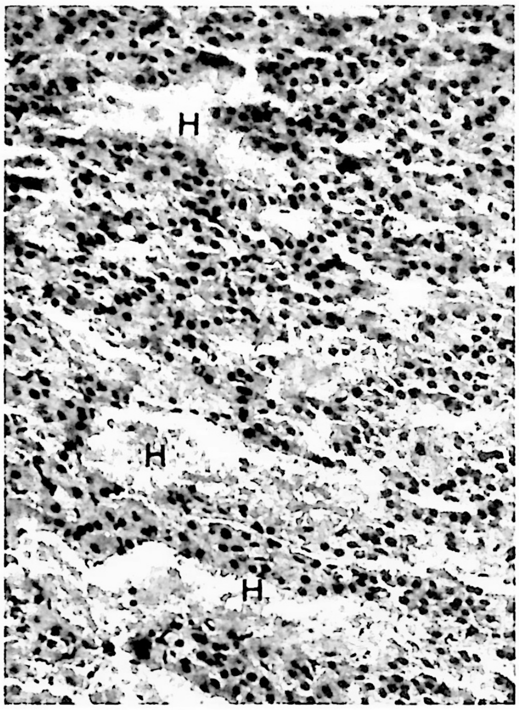

Fig. 4. *Adrenal cortex of an hypophysectomized and stressed rat, with vasodilatation and small hemorrhagic foci (H). (190 ×).*

Splanchnichotomy (Splanchnicus major). — The adrenal cortex of the left gland (neurotomy side) exhibited a structure and vascular pattern similar to the adrenal cortex of the right gland. When the rats were stressed, the behaviour of both adrenals was very different; while the right adrenal showed a vascular reaction comparable to that presented by the adrenals of the stressed control rats, the left gland only showed vasodilatation in the inner layer of the ZF and in the ZR (fig. 5). Capillary bed: 1.4 %.

Vagotomy. — Vagotomy caused a hypertrophy of the adrenal cortex observable macroscopically as well as microscopically, but the structure was no different from the right control gland. The rats with neurogenic stress showed an intense vascular reaction not only in the control adrenal (right) but also in the left. The vagotomized adrenal exhibited more abundant and extense hemorrhages and edema (fig. 6). Hemorrhage area: 3.5 %, capillary bed: 6 % in the left adrenal cortex, and in the right one 2.8 % and 5.8 %, respectively.

Discussion

The adrenals were enucleated before autotransplantation to favour their regener-

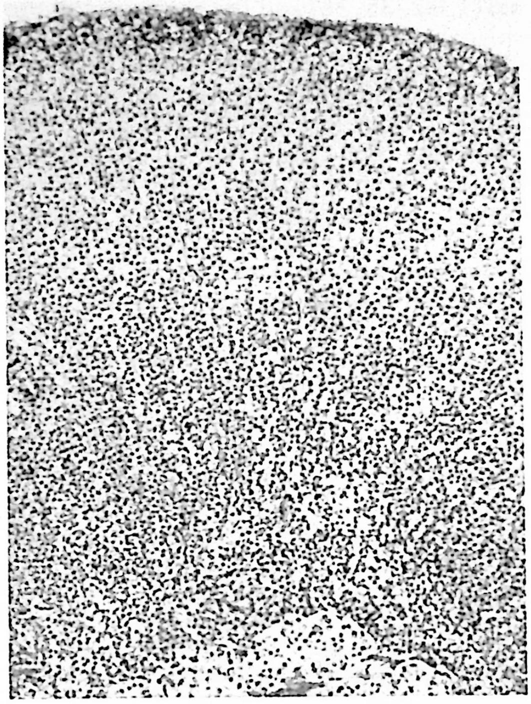

Fig. 5. *Adrenal gland of a splancnicotomized (ipsilateral side) and stressed rat.*
Only a slight vasodilatation is observed in zona fasciculata interna and reticularis. (75 ×).

Fig. 6. *Adrenal gland of a vagotomized rat on the same side.*
It shows a more widespread vasodilatation, edema and hemorrhages in zona fasciculata interna and reticularis (H) (75 ×).

ation; otherwise the necrotic tissue (the medulla and the greater part of the adrenal cortex) would be slowly removed. The regeneration takes place from Bachmann's subcapsular blastem and glomerulosa (13) and, when the animals were sacrificed, this was complete or, at least, very advanced. The difference between the transplanted and enucleated adrenals consisted that the latter were left in their place and with their innervation. In the grafted adrenals all the adrenal nerves were out while those in the enucleated gland were not. It is true that in enucleating the medulla and the greater part of the cortex the intracortical nerve fibres were destroyed, but they remained intact in the outer zone of the cortex, so that fibre regeneration was possible during the recovery time and this could explain its different behaviour in comparison with the autotransplanted animals.

The vascular reaction provoked by neurogenic stress in the rat adrenals was very conspicuous in the strain of rat utilized in our experiments, although in other strains it is different (10, 11).

Role of the hypophysis. — The present results have shown that the hypophysis plays a slight role in the adrenovascular reaction to neurogenic stress. In fact, the hypophysectomized animals showed a similar reaction to that of the stressed controls, which indicates that the adrenovascular lesions were due to a neural and not to a humoral factor.

Adrenal transplantation. — The results obtained with the adrenal autograft confirm that the autonomic nervous system is responsible for the adrenovascular lesions. In fact, the suppression of the adrenal innervation abolishes the vascular reaction of the gland to stress.

Adrenal enucleation. — A confirmation of the results of the previous experiment was obtained by adrenal enucleation. In these cases, the regenerated nerve fibres caused a vascular response in the adrenal, similar to that in the controls. This experiment also, showed that the adrenal medulla is not necessary for the adrenovascular reaction to neurogenic stress.

Vagal role. — The adrenal denervation prevents, as shown above, the adrenovascular alterations following the application of a neurogenic stressor. To know which of both systems, sympathetic or parasympathetic, is the responsible for such a result, it was necessary to suppress either the vagus or the splanchnicus nerve.

The parasympathetic innervation of the adrenal, that correspond to the vagus nerve (25, 26, 28), plays and important role in the corticoadrenal function (9, 13, 18, 19) but, according to the present results, its significance in the adrenovascular response to neurogenic stressors seems negligeable since vagotomy did not decrease such reaction, but on the contrary, it was augmented. This result should not be interpreted as a consequence of a partial regeneration of the vagal fibres because at necropsy the separation of both ends of the sectioned nerve was checked, their regeneration requiring, on the other hand, more time than the survival period of these animals (6, 16, 21, 31).

Role of the sympathetic innervation. — The rich adrenergic innervation of the adrenal (1, 22, 35, 36) and the responses of the splanchnicus major to painful stimulation (2, 29), favor the hypothesis that the sympathetic innervation of the adrenal may have an important role in the adrenovascular alterations following the action of a neurogenic stressor. Our results have confirmed such hypothesis since the transection of the splanchnicus major nerve prevents this vascular reaction of the adrenals. The vasodilatation found in the inner ZF and ZR on the side of splanchnicotomy was possibly originated from impulses that reached the adrenal via the splanchnicus minor, which also innervates this gland (3, 20, 22), and perhaps also via the lumbar sympathetic nerves (27).

Resumen

La aplicación de un estímulo doloroso a ratas, atadas a la mesa de operaciones, provoca notables alteraciones en la corteza suprarrenal (vasodilatación, edema, pequeños focos de hemorragia). Ni la hipofisectomía ni la vagotomía abdominal modifican esta respuesta, y por el contrario, la esplacnicotomía la suprime casi por completo. Estos resultados muestran que las alteraciones vasculares de la suprarrenal, causadas por un estrés neurógeno, no se deben a una respuesta hipofiso-humoral, sino a la acción del simpático.

Palabras clave: Suprarrenal, Lesiones suprarrenales, Estrés neurógeno, Denervación suprarrenal.

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