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Vagal Influence on the Adrenocortical Function of the Rat*

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The vagal influence on the fasciculata's function was studied in 23 Wistar male rats. The corticoadrenal function was evaluated by means of karyometric and histological studies. After vagotomy, the fasciculata of the left adrenal (operated side) showed a significant increase of the nuclear area in comparison with the right (control) side. This side difference was maintained in the stressed rats. In these animals the nuclear area did not increase significantly in either of the two adrenals. These results lead to the following conclusions: the vagus nerve, in normal conditions, has an inhibitory influence on the adrenal cortex; the vagal participation in the corticoadrenal response to a neurogenic stressor is meagre; the inhibitory vagal action on the fasciculata must be direct since the corticoadrenal modifications were unilateral, whereas, if the vagal influence were exerted through the hypophysis, the adrenal reaction should be bilateral; and, finally, the participation of the vagus nerve in the adrenal vascular disorders, which appeared in the stressed rats, seems to be insignificant since both glands, vagotomized and non vagotomized, showed a similar appearance.

Key words: Adrenal cortex, Vagotomy, Vagal control.

The presence of both parasympathetic ganglion cells (21, 22, 23, 32) and cholinergic terminals (20, 32, 37) in the adrenal cortex suggests that vagal innervation influences adrenocortical function. But in spite of the abundant papers dedicated to the study of the action of ACTH on the adrenal cortex, there are few studies on the regulatory role of the vagus upon this gland. Some of them recognize only an indirect influence; that is, the vagus nerve would bring visceral information to the autonomic centres that would influence ACTH secretion. Other authors (16, 30, 34) recognize a direct action of the vagus on the adrenocortical function, although there are diverse opinions as to the action mechanism as well as its effects (2, 6, 15, 27, 28, 34). Such different results were due, at least in part, to the diverse methods employed to suppress the vagal innervation of the adrenal (3, 9, 14, 18, 34). The following experi-

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ments were carried out to clarify the role of the vagus nerve in the control of the adrenocortical function, both in normal as well as in stressed animals.

Materials and Methods

Twenty-three male Wistar rats (Biocentre, Barcelona), weighing between 200 and 250 gm, were divided into two groups; control and vagotomized.

After a 48-hour fast (in order to facilitate manipulation of the hollow abdominal viscera), the animals were anaesthetized by i.p. Penthotal R (135 mg/100 g B.W.). Following medial laparotomy, the distal portion of the oesophagus was exposed by separating the liver portions which hide it and by gently pulling at the stomach distally. With the aid of a surgical microscope (OPMi I. Zeiss), 3 mm of the anterior vagal trunk was exposed and a 2 mm fragment of it was removed at the cardiac level. The viscera were immediately returned to their original position and the incision sutured. Animals of the control group underwent the same surgical procedure, except for the nerve section.

One month was allowed to elapse between surgery and decapitation. During this time, all of the animals were kept under the same conditions (food and water *ad libitum*, natural light/dark sequence, and 22 ± 1° C temperature). Recovery from surgery was satisfactory in all cases.

Before the sacrifice, the animals were grouped according to stress application as follows: Control group: unstressed rats ($n = 6$), and stressed rats ($n = 5$). Vagotomy group: unstressed rats ($n = 5$); and stressed rats ($n = 7$).

Stress, neurogenic in nature, was applied by restraining the animal in prone position and pricking its back for 5 min (10). Half an hour later, the animals were decapitated.

Nerve trunk section was confirmed during the post-mortem examination; both adrenal glands were removed, fixed in 10% formalin and processed for paraffin inclusion; thereupon 7 μm sections were made and stained with hematoxylin-eosin.

The evaluation of the adrenal cortex function was carried out by using a karyometric method based on the known fact that every change in cell activity is followed by a parallel change in nuclear size (13). The nuclear contour of cells from the zona fasciculata (1, 19, 36) was drawn by means of a camera lucida (Zeiss); 100 nuclei of both the outer and inner fasciculata layers (35) were used. Their area was measured with a 9864 H.P. interfaced in a 9830A H.P. computer. Statistics were carried out by using «paired t test», and $2p < 0.05$ was considered as significant.

Parenchymal (relative thickness of different layers) and vascular changes of the adrenal cortex were also taken into consideration.

Results

Control group. — The karyometric study of the unstressed rats revealed no significant differences between the left and the right adrenals for either the outer or the inner fasciculata layer ($2p < 0.5$ for both), nor were there significant differences between outer and inner layers of both fasciculatae ($2p < 0.30$ for the left and $2p < 0.35$ for the right) (table I).

The structure of the adrenal fasciculata was that described in the classic papers: zona fasciculata occupied little more than 2/3 of the total cortex thickness; in the zona fasciculata, the outer layer was nearly twice as large as the inner one.

Rats undergoing neurogenic stress. Karyometric study. Not significant differences were found either between adrenals or between layers ($2p < 0.5$ and $2p <$

Table I. *Results of the karyometric study.*
Mean nuclear areas (μ^2) ± standard deviation. NS = Non stressed, S = Stressed, L = Left adrenal, R = Right adrenal, OL = outer layer, IL = inner layer.

		L/OL	R/OL	L/IL	R/IL
Control	NS	35.76 ± 6.12	34.17 ± 5.75	30.31 ± 7.45	29.80 ± 7.47
	S	41.72 ± 8.60	40.23 ± 8.15	34.31 ± 8.20	32.99 ± 8.91
Vagotomy	NS	43.04 ± 3.75	29.38 ± 4.74	36.90 ± 4.30	25.25 ± 2.51
	S	46.74 ± 6.59	32.96 ± 6.14	39.18 ± 6.95	29.91 ± 6.16

0.30, respectively). The increase caused by the neurogenic stress was not significant in any of the studied zones ($2p < 0.30$ for the outer left and $2p < 0.25$ for the outer right, and $2p < 0.5$ for the inner right and $2p < 0.5$ for the inner left (table I).

Histological study. The most noteworthy changes after the application of the neurogenic stress were: an increase in the outer/inner layer ratio; vasodilatation in both adrenals, operated and control; and, in 4 of the 5 individuals, hemorrhagic foci located, bilaterally, in the outer zone of the fasciculata.

Vagotomized rats. — Unstressed. The histological study showed a global hypertrophy of the adrenal cortex in the gland without vagal innervation in comparison to the contralateral one and to the adrenals of the intact rats. This hypertrophy was greater in the fasciculata zone and specially in its-outer layer.

The karyometric study confirmed the histological observations, showing a very significant increase on the nuclear area of the fasciculata cells in the vagotomized adrenals in comparison to the contralateral ones ($2p < 0.005$ for both layers). This difference was similarly significant with respect to the adrenals of the control group (table I). The nuclear area of the fasciculata in the non vagotomized side was smaller than that of the control group (−14% for the outer and −15% for the inner layer).

Rats undergoing a neurogenic stress. The histological structure of the adrenal cortex showed little variations after the application of the neurogenic stress, persisted the greater amplitude of the fasciculata in the side of vagotomy and the increased ratio outer/inner fasciculata.

The vascular disorders were of the same extent in both vagotomized and control adrenals and similar to those showed by the intact animals under the action of the same stressor.

Karyometric study. The neurogenic stress provoked a non significant increase of the nuclear size of the fasciculata cells in both adrenals (table I) but while in the inner fasciculata this increase was of the same proportion in both, vagotomized and non vagotomized rats, in the outer was only the half in the vagotomized group. These changes did not alter the significant differences between the fasciculata of both sides vagotomized and non vagotomized. The nuclear size in the vagotomized fasciculata was significantly larger than that of the non vagotomized. ($2p < 0.05$ for both layers). There were no significant differences between layers of the same fasciculata ($2p < 0.10$ for the right and $2p < 0.45$ for the left).

Discussion

The most noticeable feature of the above mentioned results was the hypertrophy of both layers —inner and out-

er— of the adrenal fasciculata of the operated side. This hypertrophy may have been caused by: a) suppression of the visceral information transmitted by the vagus nerve to the autonomic centres; b) elimination of inputs to the adrenal cortex; or c) a combination of both mechanisms a and b. The first hypothesis, the most widely followed, especially when the vagotomy was performed at the cervical level, maintains that suppression of cardiovascular inputs to higher nervous centres provokes an increase in the plasmatic levels of 17-hydroxycorticoids (8, 9). Other authors (12, 14, 31) consider the interruption of the afferences from the gastrointestinal tract as responsible for the variation in the secretion of ACTH. Supporting these assertions, BORISOVA (4) observed a depletion of Gomori-positive material in the hypophysis after stimulation of the vagus nerve. In our case these mechanisms did not come into consideration because the vagal section was unilateral and subdiaphragmatic. Not even central afferences from the adrenal (17, 24-26, 33) could explain this central effect upon the adrenal cortex; which is that, if the vagal influence were mediated through the hypophysis, then both adrenals should manifest a similar state. But in our case, the animals with unilateral, subdiaphragmatic, vagotomy only presented hypertrophy in the adrenal gland of the vagotomized side. Consequently, these results can be explained only by the second hypothesis; which is that, there is a vagal input to the adrenal cortex that influences its function. This vagal influence is inhibitory, although the vagotomized adrenal cortex shows a significant hypertrophy when the vagal action is suppressed. This phenomenon indicates that, normally, the central nervous system performs an inhibitory action upon the adrenocortical function. Moreover, this conclusion is corroborated by the results reported by other authors (10, 29, 30), according to whom the cerebral cortex has an inhibitory influence on the adrenal cortex, mediated through the autonomic nervous system and not through the hypophysis.

The fasciculata of the nonvagotomized adrenal showed, in contrast with the vagotomized adrenal, a decrease of the nuclear area. This fact can be interpreted as a result of a central inhibition caused by the higher plasmatic level of corticosteroids subsequent to the left corticoadrenal hypertrophy.

The small response of the adrenal cortex, to the neurogenic stress contrasts, not only in the vagotomized but also in the intact rats, with the considerable hypertrophy of the fasciculata and the notable increase of the nuclear size following the action of a humoral stress (35, 36) or by the injection of ACTH (1, 7). On the other hand, the similar response to the neurogenic stress by the fasciculata cells of both sides, vagotomized and intact, indicates that the vagus nerve does not play an important role in the reaction of the adrenal cortex to this neurogenic stress. The sligthly smaller response of the vagotomized rats to the neurogenic stress can be explained by the already existent hypertrophy in the vagotomized adrenal.

Finally, the vagal participation in the vascular disorders of the adrenal cortex —manifested itself after application of the neurogenic stressor— (5, 11), seems to be of little importance because the differences between the normal and the denervated adrenals were inappreciable.

Resumen

La vagotomía subdiafragmática izquierda provoca en la rata una hipertrofia de la corteza suprarrenal del lado de la vagotomía y un significativo aumento del tamaño nuclear de sus células. La aplicación de un estrés neurógeno provoca en las ratas intactas y en las vagotomizadas un aumento no significativo del tamaño nuclear de las células de la zona fascicu-

lada. Las alteraciones vasculares también son similares en ambos grupos de animales. Estos resultados llevan a las siguientes conclusiones: el vago, en condiciones normales, ejerce un efecto inhibidor sobre el cortex suprarrenal; su participación en la respuesta suprarrenal ante la acción de un estrés neurógeno es escasa; y tampoco parece jugar un papel importante en los trastornos vasculares que aparecen en la suprarrenal como consecuencia de la acción del citado estrés.

Palabras clave: Corteza suprarrenal, Vagotomía, Control vagal.

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