Differential Response to Aminergic Stimuli and Biological Behavior of Growth Hormone Secreting Pituitary Adenomas

Guillermo Fanghanel, Oscar Larraza, Martha Villalobos, Leticia Fanghanel, Marcos Velasco and Francisco Velasco

ABSTRACT: Growth hormone (GH) serum levels in response to the administration of aminergic drugs and thyroliberine (TRH) were determined in a group of 34 acromegalics. Administration of bromocriptine (10 mg single oral dose) was followed by a decrease in GH below 60% control values in 35% of the cases. Administration of diazepam (10 mg single oral dose) to those cases not responding to bromocriptine induced a decrease in GH in 58% of the cases and an increase in GH in 42%. Administration of cyproheptadine (24 mg/day for one month) to those cases not responding to bromocriptine or with increased GH after the administration of diazepam, decreased GH in 75%, while increased GH in 25% of the cases. TRH 200 μg single I.V. dose induced increase of 128% GH basal level in 65% of cases (TRH positive) which correlated with more benign clinical course, decreased GH levels in response to bromocriptine, increased PRL levels, PRL-GH mixed secreting adenomas in immunohistochemistry studies, presence of granulated cells in electron microscopy studies and normalization of GH in the majority of surgically treated cases. By contrast, TRH negative cells correlated with aggressive tumor growth, lack of response to bromocriptine, normal PRL levels, pure GH secreting adenomas by immunohistochemistry, poorly granulated cells and lack of response to surgical treatment. Results suggest that there is more than one type of acromegaly that might be distinguished by the aminergic control on GH secretion.

RÉSUMÉ: Réponse différentielle à des stimuli aminergiques et comportement biologique des adénomes pituitaires secrétant de l'hormone de croissance Les taux sérique d'hormone de croissance (GH) ont été étudiés chez 34 acromégales en réponse à l'administration de substances aminergiques et de thyréostimuline (TRH). L'administration de bromocriptine (dose orale unique de 10 mg) a été suivie d'une baisse de la GH de 60% par rapport aux valeurs de contrôle dans 35% des cas. L'administration de diazepam (10 mg en dose orale unique) aux patients qui n'avaient pas répondu à la bromocriptine, a entraîné une diminution de la GH dans 58% des cas et une augmentation dans 42%. L'administration de cyproheptadine (24 mg/jour pendant un mois) aux patients n'ayant répondu ni à la bromocriptine, ni au diazepam, augmenta la GH dans 75% et la diminua dans 25% des cas. La TRH administrée à raison de 200 mg intraveineux provoqua une augmentation de 128% du taux basal de GH dans 75% des cas (positivité à la TRH). La réponse positive à la TRH était en corrélation avec une évolution clinique plus bénigne, avec réponse à la bromocriptine, augmentation des taux aux de PRL (adénomes mixtes), présence de cellules granulées à la microscopie électronique et normalisation de la GH dans la majorité des cas traités chirurgicalement. Par contre, la négativité à la TRH était accompagnée d'une croissance agressive, d'une absence de réponse à la bromocriptine, de taux de PRL normaux (adénomes somatotropes purs), de cellules peu granulées et d'absence de réponse au traitement chirurgical. Ces résultats suggèrent qu'il existe plus d'un type d'acromégalie.

Can. J. Neurol. Sci. 1990; 17:78-82

In normal conditions, growth hormone (GH) secretion is regulated by hypothalamic hormones: somatostatin (GH-IH)[1,2] and somatocrinine (GH-RH).[3,4] In turn, GH-RH secretion in the hypothalamus is under the control of aminergic (dopamine and norepinephrine) and serotonine neurons in other nervous structures, while there is no evidence of aminergic control on GH-IH secretion.[5] In acromegaly, however, GH-IH secretion is under the control of dopamine and γ-aminobutyric acid (GABA), while GH-RH is regulated by serotonine and norepinephrine but not by dopamine.[6]

Thyroliberine (TRH), a hypothalamic hormone that in normal conditions does not play a significant role in GH secretion, may become a powerful stimulus for increasing GH serum levels in acromegaly.[7,8] Such differences in regulation of GH secretion in normal conditions and acromegaly, have been used for diagnostic and therapeutic purposes.[9,10,11,12] Changes in GH

From the Hypothalamus-Hypophysis Clinic, Hospital General de Mexico, S.S. and Division of Neurophysiology, Scientific Research Department. IMSS Mexico, D.F. Mexico

Reprint requests to: Dr. Francisco Velasco, Division of Neurophysiology, Scientific Research Dept., National Medical Centre, IMSS, P.O. Box 73-032, Mexico, D.F. Mexico

serum level in response to the administration of amines and peptides agonists vary a great deal among acromegalics, suggesting that acromegaly results from the disfunction of more than one physiological mechanism controlling GH secretion, which in turn may determine the different biological behavior seen in GH secreting pituitary adenomas.[8]

In the present report, differential responses to aminergic and peptidergic stimuli of a group of acromegalics were studied and the results tested against the biological behavior of the tumor.

Materials and Methods

The study was carried out on thirty-four patients (22 male and 12 female), with ages between 14 and 60 years and with clinic diagnosis of acromegaly. Symptoms of acromegaly were noticed for the first time between 5 months to 16 years before the admission to the study. The diagnosis was confirmed by radiological (plain skull X rays, lineal tomography of the sella turcica and CT scan) and hormonal studies (radioimmunoassay determination of GH, PRL, LH and FSH). Afterwards, patients underwent the following protocol of study: in all studies, except cyproheptadine, patients were hospitalized at least 24 hours in advance, being on bed rest and fasting for periods between 8 to 11 hours prior to the administration of the drugs which were given between 7:00 and 9:00 A.M. Blood samples were taken through an indwelling catheter in the antecubital vein maintained with a slow infusion of normal saline and kept at -4°C temperature until duplicated determinations of GH, PRL and glucose in all samples were performed, according to the technique described elsewhere.[7] Intra-assay variations for GH in our laboratory were 1.040 ± 0.220 ng/ml and inter-assay variations of 2.008 ± 0.640 ng/ml.

The 34 patients received bromocriptine, a dopamine agonist[13,14,15] in a single oral dose of 10 mg. Blood samples were taken at 15 min. before and 2, 3, 4 and 5 hours after administration of bromocriptine.

Twelve patients, among those who received bromocriptine and did not have significant decrease in GH serum levels in response to the drug (see results), received a single oral dose of 10 mg of diazepam, (a GABA agonist),[16,17] while blood samples were taken before, and 15, 30, 45 and 60 min. after diazepam administration, in the same way as that for the bromocriptine test. The diazepam test was performed at least one week after the bromocriptine test. Four patients that did not have a decrease in GH serum levels after the administration of either bromocriptine or diazepam received cyproheptadine, a potent serotonine antagonist, although also having anticholinergic and antihistaminic action,[18,19] in a daily oral dose of 24 mg for a minimum of a month. Blood samples for GH determination were taken before cyproheptadine was started and every 15 days thereafter. Blood samples were taken also after a night of rest and fasting of 8 to 12 hours, but patients were neither hospitalized, nor was an indwelling catheter placed to take blood samples for this test.

All patients were surgically treated and the surgical approach was decided on the basis of tumor extension and optic nerve involvement: 19 were treated through trans-sphenoidal approach, and 15 through subfrontal approach. The surgical specimen was studied with electron microscopy and immunohistochemistry according to the techniques described elsewhere.[20,21] A post operative evaluation was carried out four months to one year later using clinical radiological and GH and PRL determinations as for the preoperative evaluation. Changes in GH serum levels comparing the control values with the value that presented the maximum modification after bromocriptine, diazepam, cyproheptadine and TRH were determined. Cases that presented significant decrease or increase in GH serum levels after the administration of one of the drugs (see results) were grouped and the mean value, standard deviation and standard error of GH changes for the group were calculated. Significance of changes was determined through paired student "t" test.

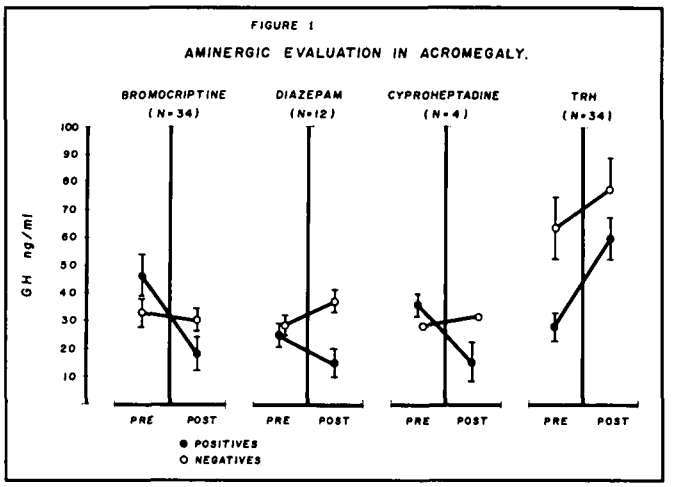

Figure 1 — Different response to aminergic and peptidergic stimulation in the group of 34 acromegalics. Filled circles indicate the mean value of the group that had positive responses and empty circles the group with "negative" responses. The bars on top of circles indicate the standard error. Notice that TRH negative group had higher levels of GH to start with, which may lead to a misinterpretation of the results when the response is estimated as the percent increase over basal levels. However, even if results are estimated as increase in ng/ml, there is still a significant difference between positive and negative groups.

Table 1: Differential Response to Aminergic Stimulation in Acromegalic Patients

	Bromocriptine +			Bromocriptine −			Diazepam +			Diazepam −			Cyproheptadine +			Cyproheptadine −		
	GH-Pre	GH-Post	%	GH-Pre	GH-Post	%	GH-Pre	GH-Post	%	GH-Pre	GH-Post	%	GH-Pre	GH-Post	%	GH-Pre	GH-Post	%
$\bar{x}$	46.1	17.4	−63	31.8	30.1	−2.2	25.1	15.0	−33	29	38	+30	35.5	16.5	−55	27.5	32	+16
SD	26.1	14.1	18	18.0	15.3	17.4	10.1	4.5	16.1	3.0	7.2	18	5.9	7.6	14			
SE	7.5	4.0	4.5	3.8	3.2	3.7	3.8	1.7	6.1	1.3	3.2	8	3.4	4.4	8			
t	6.03			1.32			2.99			3.45			9.89					
p	<0.001			NS			<0.01			<0.05			<0.01					
n=	12			22			7			5			3			1		

Mean GH serum levels in ng/ml for each group pre and post different aminergic stimuli, as well as the percent of increments and decrements are presented. The significance of changes for each group are given at the foot of each column.

https://doi.org/10.1017/S0317167100030092 Published online by Cambridge University Press

Table 2: TRH Response and Biological Behavior

	Symptoms Evolution (Years)	Tumor Size Grade	Number	Bromocriptine		Cytoplasmic Granules		Immunohistochemistry		Surgical Outcome	
				+	−	Dense	Sparced	GH-PRL	GH	Good	Poor
TRH+	$\bar{x}$ 4.37	I - II	18	9	9	9	9	14	4	15	3
	± 3.71	III - IV	4	1	3	2	1	1	3	1	3
TRH−	$\bar{x}$ 1.96	I - II	1	1	0	0	1	0	1	0	1
	± 0.95	III - IV	11	1	10	0	11	2	9	0	11
Significance of Differences		Chi Square	14.157	1.698		11.437		8.002		13.694	
		P	<0.001	NS		<0.01		<0.05		<0.001	

Cases have been divided in TRH (+) and TRH (−) groups and each group in grade I-II and III-IV subgroups. The differences between the four subgroups have been evaluated by chi-square test and the results presented at the bottom lines with the significance of changes.

The response to TRH administration was compared with the clinical course and tumor size, response to bromocriptine, electron microscopy and immunohistochemistry, levels of PRL and outcome after surgical treatment. The statistical significance of differences between TRH positive and negative groups and biological behavior of the tumor was evaluated through the chi square test.

Results

Results of aminergic stimulations are presented in Table 1 and Figure 1. Patients may be classified according to their response in the following categories:

In 12 out of 34 cases bromocriptine induced a decrease from 50 to 96% in the basal levels of GH ($\bar{x}$ = −63.3%, significance of change $p < 0.01$). Those patients were labeled as bromocriptine positive, while the other 22 patients showed responses that varied from a decrease of 33% to an increase of 31% in the basal levels of GH ($\bar{x}$ = +2.2%, no significant change) and were labeled as bromocriptine negative.

In 7 out of 12 bromocriptine negative patients, diazepam induced a significant decrease in GH serum levels (abnormal response), that varied from 15 to 62% of the basal values ($\bar{x}$ = −32.8%, $p < 0.01$) and were labeled as diazepam positive, while the other 5 patients had a significant increase (normal response) that varied from 10 to 58% of the control value ($\bar{x}$ = +30%, $p < 0.05$) and were labeled as diazepam negative.

Cyproheptadine decreased GH serum levels in 3 out of 4 bromocriptine negative, diazepam negative patients, varying

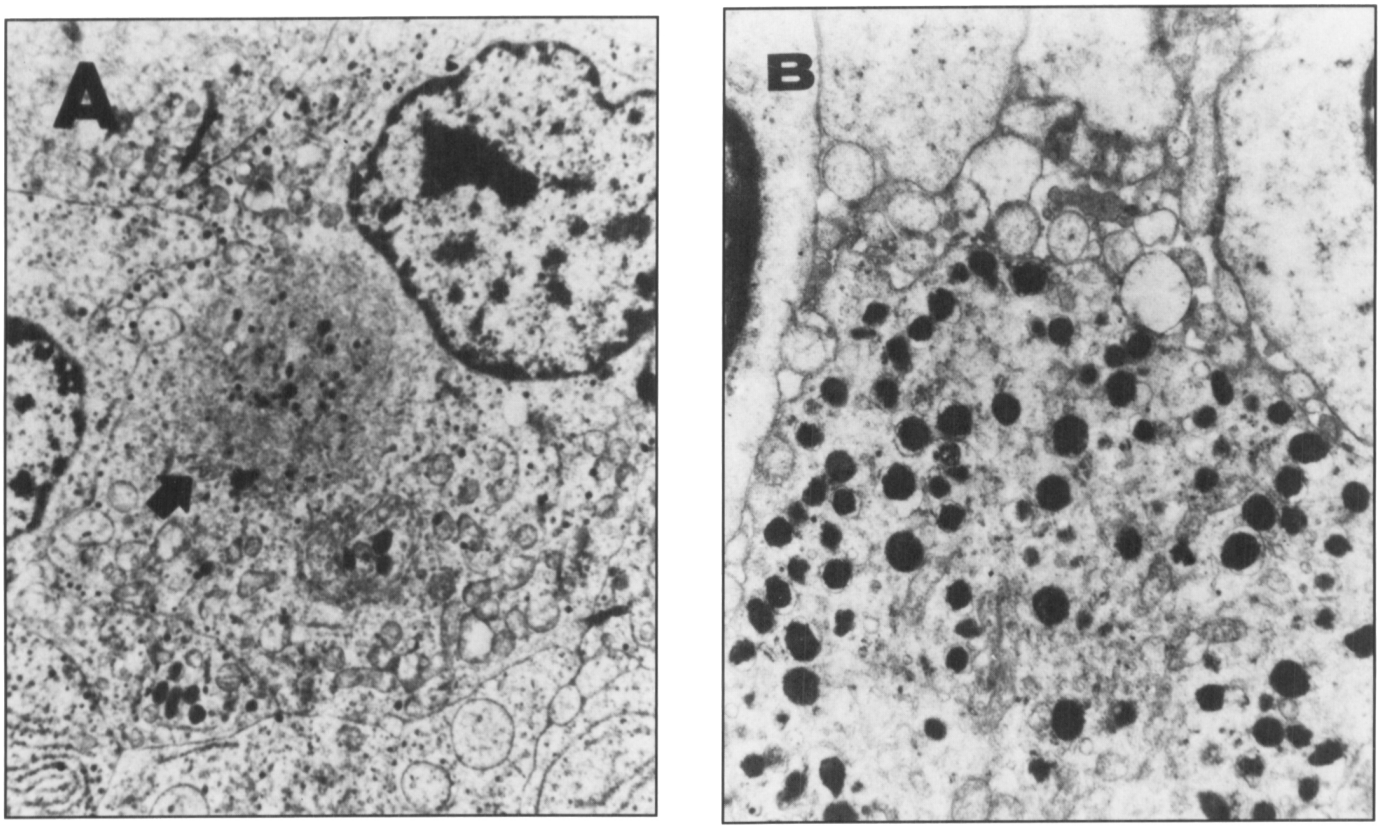

Figure 2 — Electron microscope pictures from 2 GH secreting pituitary adenomas showing: A: Sparcely granulated cell with fibrous body near the nucleus (arrow). B: Portion of a densely granulated cell with granules measuring 300 nm in average (24500X) (uranyl-acetate-lead-citrate preparation).

https://doi.org/10.1017/S0317167100030092 Published online by Cambridge University Press

from 41 to 69% the basal levels ($\bar{x}$ = −54.6%, $p < 0.01$), while GH serum levels increased in 16% in the other patient. The former 3 patients were labeled as cyproheptadine positive and the latter as cyproheptadine negative.

Administration of TRH to the same 34 patients induced increase in GH serum levels over 100% of the control value ($\bar{x}$ = +128%, $p < 0.001$) in 22 patients labeled as TRH positive, while GH increased from 0 to 37% ($\bar{x}$ = +21%, not significant) in the other 12 patients and were labeled as TRH negative. It is important to mention that TRH negative cases had GH serum levels significantly higher than TRH positive cases. Still, the increase in absolute values of GH after administration of TRH was significantly larger in TRH positive than in TRH negative cases.

Results of correlation between TRH test and clinical course plus tumor size and surgical outcome are presented in Table 2 and Figure 2. It may be seen that although TRH positive cases presented symptoms over longer periods of time (4.3 ± 3.7 years), than TRH negative cases (1.9 ± 0.9 years), 18 out of 22 TRH positive patients (81.8%) had tumors classified as stages I-II from the radiological point of view,[22] i.e.: microadenomas or intrasellar non invasive macroadenomas. In contrast 11 out of 12 TRH negative patients (91.6%) presented stages III and IV, i.e.: large invasive intrasellar or intrasellar and suprasellar tumors ($p < 0.001$). Ten out of 22 TRH positive cases (41.6%) were bromocriptine positive, while only 2 out of 12 TRH negative (16.6%) were bromocriptine positive. The difference, however, was not significant. Eleven out of 12 TRH negative (91.6%) had poorly granulated tumoral cells in electron microscopy studies, while TRH positive patients had poorly granulated cells in 8 out of 22 cases. (36.4%) being the difference between groups significant ($p < 0.01$). TRH positive cases had increased PRL levels in 20 cases (91%), TRH negative had increased PRL in 4 cases (33.3%), being the difference between groups significant ($p < 0.05$), in spite of the fact that TRH negative group had larger tumors and it is common to see increased PRL levels in large pituitary tumors due to compression of the pituitary stalk.[23] In our cases, increased GH and PRL serum levels closely correlated with the presence of a GH-PRL mixed secreting pituitary cell tumor in the immunohistochemistry studies in the same case (17 out of 21). Sixteen of the TRH positive cases (72.7%) had normalization of GH levels after surgery, while none of TRH negative cases presented normal GH levels in the post operative control study, which may be in part due to the smaller tumor size in TRH positive cases. The difference between groups was significant ($p < 0.001$).

Discussion

There are limitations inherent to the protocol of study followed in the present report, for example: the number of cases is small, particularly for the group receiving cyproheptadine; single doses of bromocriptine and diazepam were used, and it is possible that larger doses given for longer periods of time, may have induced decrease in GH serum levels in those cases herein described as bromocriptine or diazepam negative, as has been the experience with bromocriptine treatment in acromegaly.[9,10,24] The tumor size in some cases was rather large, which may interfere with the response to various stimuli by compression of the pituitary stalk and the hypothalamus. Finally, some of the drugs used for stimulation have a complicated and still debatable pharmacological mode of action, as is the case of diazepam in regard to GABA systems[16,25] and cyproheptadine in regard to serotonine versus acetylcholine and histamine.[18,19] At the side of these limitations, present results indicate that a group of acromegalics do not respond to bromocriptine and suggest that in those cases the increase in GH secretion depends on mechanisms other than the absolute or relative decrease in DOPA control either on GH-IH secretion[6] or secretion rate at the adenomatous cell itself. Some of those bromocriptine negative patients responded to the administration of a GABA agonist diazepam, decreasing the serum levels of GH, while the rest increased the levels of GH in response to the same drug. That is, considering only the effect of diazepam on GABA it seems that in some cases it decreases secretion of GH (diazepam positive), whereas in some others it increases secretion of GH (diazepam negative). The former has been reported as a characteristic response in acromegalics,[16] whereas the increase in GH in response to diazepam is the normal response. If one evaluates all cases together, no significant changes are seen in response to diazepam as has been reported by others.[17] Among those diazepam negative cases the majority responded to the administration of cyproheptadine, suggesting an overactivity of serotonine suprahypophyseal control. There are a few cases (one in our series), that do not respond to any of the above mentioned stimuli, indicating that there may be other forms of control on GH abnormal secretion in acromegaly. Unfortunately, we did not perform diazepam or cyproheptadine tests in those bromocriptine positive patients, to explore the possibility that some patients with DOPA decreased activity may also have alteration in GABA or serotonine control. For what has been mentioned, it is possible that acromegaly results from the disruption of more than one aminergic mechanism and therefore, it should be expected that medical treatment using a single drug may result in a number of failures. On the other hand, we have been impressed with the different biological behavior of the GH secreting tumors: while some patients have a rather slow growing tumor that never reaches a large size over many years, others return a few months after surgical treatment with tumor masses larger than those originally treated.[26] The analysis of the clinical course, hormone secretion and histological features of the GH secreting tumors and their response to TRH and bromocriptine administration, led to some conclusions of prognostic value. For example, the difference in biological behavior of tumors between the TRH positive and TRH negative patients was striking: while the former had benign tumors the later had aggressive ones. Admittedly, the positive or negative response to TRH is taken on an arbitrary basis of 100% increase over control GH values, and although the control values were significantly higher for TRH negative group, the difference in response to TRH between the 2 groups was rather significant. The same favorable prognostic value of a TRH positive response has been found in the prolactin secreting pituitary adenomas.[27]

It is not possible from this study to determine if this "paradoxical" response to TRH is due to a liberation of GH stored in intracytoplasmic granules of tumor cells or is the result of increased synthesis of GH in those cells; however, since it was found that intracytoplasmic granules are more abundant in the

cells of those TRH positive cases, it is possible that "paradoxical" TRH response results from the liberation of previously stored GH. *In vitro* studies in prolactin secreting pituitary adenomas in regard to PRL secretion, indicate that the positive response to TRH is probably due to an increased release of PRL from cell granules.[28] The above mentioned differences on aminergic control, as well as the contrasting biological behavior of GH secreting adenomas, support the hypothesis that acromegaly may result from different etiologies and may be considered a syndrome rather than a disease.

References

1. Brazeau P, Vale W, Burgus R, et al. Hypothalamic peptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science 1973; 179: 77-81.
2. Siler TM, Vanderberg G, Yen SS, et al. Inhibition of growth hormone release in humans by somatostatin. J Clin Endocrinol Metab 1973; 37: 632-636.
3. Schally AV, Baba Y, Nair RMG, et al. The amino-acid sequence of a peptide with growth hormone releasing activity isolated from porcine hypothalamus. J Biol Chem 1971; 246: 6647-6654.
4. Guillemin R, Brazeau P, Ohlen P, et al. Growth hormone-releasing factor from human pancreatic tumor that caused acromegaly. Science 1982; 218: 585-587.
5. MacLeod RM. Monoaminas hipotalámicas y secreción de hormonas hipofisiarias en condiciones normales y patológicas. En nuevos conceptos sobre fisiología y patología hipotálamo-hipofisiaria. (Valverde C, Fanghanel G, Mena F, eds.) Conacyt Publicaciones. (México, D.F.) 1982; 103-134.
6. Russell SM. Regulación de la secreción de la hormona de crecimiento. En nuevos conceptos sobre fisiología y patología hipotálamo-hipofisiaria. (Valverde C, Fanghanel G, Mena, F, eds.) Conacyt Publicaciones (México, D.F.) 1982; 219-242.
7. Fanghanel G, Larraza O, Arauco E, et al. Serum growth hormone and ultrastructural study of adenohypophyseal tissue in bromocriptine treated acromegalic patients. Clin Endocrinol (OXF) 1978; 4: 289-295.
8. Shibasaki T, Hotta M, Masuda A. Studies on the response of growth hormone secretion to GH-releasing hormone, thyrotropin releasing hormone, gonadotropin-releasing hormone and somatostatin in acromegaly. J Clin Endocrinol Metab 1986; 63: 167-173.
9. Thorner MO, Chait A, Aitken M, et al. Bromocriptine treatment of acromegaly. Br Med J 1975; 1: 299-303.
10. Teasdale G. Bromocriptine in acromegaly. The Lancet 1976; 1: 484-488.
11. Hanew K, Kokubon M, Sasaki A, et al. The spectrum of pituitary growth hormone responses to pharmacological stimuli in acromegaly. J Clin Endocrinol Metab 1980; 51: 292-297.
12. Pawlikowsky M, Strejozek K, Komorowski J. Effects of thyroliberine (TRH), bromocriptine and cyproheptadine on somatotropin secretion in acromegaly. Materia Médica Polona 1980; 12: 70-73.
13. Camanni F, Molinatti GM, Müller EE. Effect of five dopaminergic drugs on plasma growth hormone levels in acromegalic subjects. Neuroendocrinology 1975; 19: 227-231.
14. Carimna E, Lo Coco R, Lanzara P, et al. Effect of the acute administration of bromocriptine, haloperidol and pimozide on plasma GH levels in acromegaly. Acta Endocrinol (Copenh) 1979; 91: 609-613.
15. Fedele D, Molinar M, Muggeo M, et al. Bromocriptine acute effect on insulin, glucagon and growth hormone levels in acromegalic patients. J Endocrinol Invest 1980; 3: 149-153.
16. Kannan V. Diazepam test of growth hormone secretion. Hormone Metabolism Research 1981; 13: 390-393.
17. Zaccaria M, Giordano G, Ragazzi E. Lack of effect in diazepam administration on GH and PRL secretion in normal and acromegalic subjects. J Endocrinol Invest 1985; 8: 167-172.
18. Nakai Y, Imura H, Sakurai H. Effects of cyproheptadine in human growth hormone secretion. J Clin Endocrinol Metab 1974; 38: 446-449.
19. Smythe GA, Lazarus L. Suppression of growth hormone secretion by malatonine and cyproheptadine. J Clin Invest 1974; 54: 116-119.
20. Kovacs K, Corenblum B, Sirek AM, et al. Ultrastructural morphometry in cell adenomas of human pituitary. J Clin Path 1976; 29: 250-255.
21. Mc.Comb DJ, Kovacs K. Ultrastructural morphometry of sparsely granulated prolactin cell adenomas of the human pituitary. Acta Endocrinol (Copenh) 1978; 89: 521-526.
22. Vezina JL, Nelson B. Prolactin secreting pituitary microadenomas. Am J Radiol 1974; 120: 46-50.
23. Martínez-Campos A, Cornejo J, Garza Flores J, et al. Dysfunction of dopaminergic regulation of prolactin in patients with functioning and non functioning pituitary adenomas and craneopharyngeomas. Fertility and Sterility 1985; 44: 472-477.
24. Cassar J, Mashiter K, Joplin GF. Bromocriptine treatment of acromegaly. Metabolism 1977; 26: 539-545.
25. Ajlouini M. Failure of diazepam to affect growth hormone and prolactin in acromegalics. Horm Res 1983; 18: 186-190.
26. Velasco F, Valasco M, Jiménez O, et al. Prognostic factors in the surgical treatment of hypophyseal adenomas. Arch Invest Med (Mex) 1985; 16: 59-73 (Suppl 3).
27. Müller EE, Cavagnini F, Martínez-Campos A, et al. Dynamic testing of prolactin and growth hormone secretion in patients with neuroendocrine disorders. Acta Endocrinol (Copenh) 1984; 107: 155-159.
28. Larraza O, Valverde C, Fanghanel G, et al. Morphofunctional correlative analysis of prolactin, growth hormone and thyrotropin secretion in pituitary neoplasia. *In*: Prolactin secretion. A multidisciplinary approach. (Mena F, Valverde C, eds.) Academic Press 1983; 371-392.