Functional glutamate receptors in a subpopulation of anterior pituitary cells

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ABSTRACT **We have studied the effects of glutamate receptor agonists on the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) of single rat anterior pituitary (AP) cells. Ionotropic (NMDA and kainate/AMPA) and, to a smaller extent, metabotropic glutamate receptors were both present in all the five AP cell types, defined by the hormone they store. Cells within all the types responded also to thyrotropin-releasing hormone (TRH). Alternative typing by the response to four well-established hypothalamic releasing hormones (HRHs), GHRH, GnRH, CRH, and TRH, was performed. One-third of the cells were not sensitive to any HRH, another third were sensitive to only one HRH, and the last third were sensitive to more than one HRH, frequently to all four. Only the cells responding to TRH showed functional glutamate receptors. Superimposed to the above association, the strongest responses to glutamate were found in the cells responsive to multiple HRHs. These results suggest that glutamate may act, by a nonsynaptic mechanism, as a new releasing factor for one or, like TRH, several AP hormones. Coexpression of glutamate and TRH receptors in the subpopulation of cells responsive to multiple HRHs might have a functional meaning, perhaps related to phenotypic plasticity and long-term regulation of hormone secretion by the anterior pituitary.—Villalobos, C., Múñez, L., García-Sancho, J. Functional glutamate receptors in a subpopulation of anterior pituitary cells. *FASEB J.* 10, 654–660 (1996)**

Key Words: hypothalamic releasing hormones · thyrotropin-releasing hormone · NMDA

THE ANTERIOR PITUITARY (AP)[2] contains at least five different cell types that are able to secrete growth hormone (GH), prolactin (PRL), gonadotropins (FSH and LH), adrenocorticotropin (ACTH), and thyrotropin (TSH), respectively. Secretion is regulated mainly by the hypothalamus via releasing and inhibiting factors, which reach the pituitary through the hypophyseal portal system. The best-known hypothalamic releasing hormones (HRHs) are GHRH, CRH, and GnRH, which stimulate the secretion of GH, ACTH, and gonadotropins, respectively, and TRH, which stimulates secretion of TSH, PRL, and GH (1, 2). HRHs elicit a $[Ca^{2+}]_i$ increase in their cell targets, and this is not surprising as Ca^{2+} acts as second messenger in pituitary stimulus-secretion coupling (3–6).

Glutamate, the main excitatory neurotransmitter in the brain, has been reported to induce secretion of several pituitary hormones in vivo (7–9). These effects have been attributed to actions on the hypothalamus, where the presence of glutamate receptors is well documented (9). However, glutamate also induces hormone secretion in isolated AP cells (9–13). Reports on the presence of glutamate receptors in the adenohypophysis are controversial. Whereas the presence of non-*N*-methyl-D-aspartate (NMDA) -type glutamate receptors at the anterior pituitary has been suggested by immunocytochemical assays (14), glutamate binding was detected at the hypothalamus and the posterior pituitary, but not at the anterior pituitary (15).

Stimulation of glutamate receptors in neural cells elicits an increase of the cytosolic free calcium concentration ($[Ca^{2+}]_i$), which may be due to (16–18): *1)* Ca^{2+} entry through the NMDA channel complex; *2)* Ca^{2+} entry through voltage-gated Ca^{2+} channels recruited on depolarization by stimulation of other ionotropic glutamate receptors; *3)* Ca^{2+} release from intracellular stores secondary to stimulation of metabotropic receptors. In a recent study with GH_3 cells, a cell line derived from rat anterior pituitary, we found that glutamate and other acidic amino acids were able to elicit an increase of $[Ca^{2+}]_i$. However, this effect was not reproduced by selective glutamate receptor agonists. The actions of glutamate in GH_3 cells were secondary to glutamate entry through a high-affinity electrogenic glutamate transporter; this transport-mediated depolarization leads to activation

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[2]Abbreviations: AP, anterior pituitary; GH, growth hormone; ACTH, adrenocorticotropic hormone or corticotropin; TSH, thyroid-stimulating hormone or thyrotropin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PRL, prolactin; HRH, hypothalamic releasing hormone; GHRH, growth hormone-releasing hormone; GnRH, gonadotropin-releasing hormone or LHRH; CRH, corticotropin-releasing hormone; NMDA, *N*-methyl-D-aspartate; KA, kainate; QA, quisqualate; AMPA, α-amino-3-hydroxy-5-methyloxazole-4-propionic acid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; trans-ACPD, trans-1-amino-1,3-cyclopentanedicarboxylic acid; $[Ca^{2+}]_i$, cytosolic free calcium concentration.

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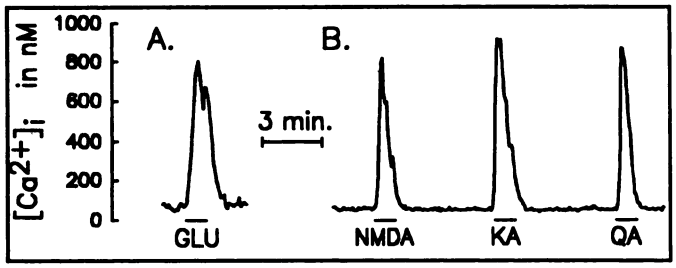

Figure 1. Effects of glutamate (*A*) and glutamate receptor agonists (*B*) on $[Ca^{2+}]_i$ of a representative cell. The concentrations of glutamate (GLU), NMDA, kainate (KA), and quisqualate (QA) were 100, 100, 50, and 1 μM, respectively.

of voltage-gated Ca^{2+} channels (19). Here we find that, in contrast to the above results, glutamate receptor agonists produce an increase of $[Ca^{2+}]_i$ in a subpopulation of rat pituitary cells maintained in primary culture. Because such a system is composed of several different cell types, an additional effort was made to identify the cell targets of glutamate receptor agonists in the anterior pituitary.

EXPERIMENTAL PROCEDURES

Anterior pituitary cells were prepared from 8- to 10-wk-old male Wistar rats as described by Dobson and Brown (20). Cells were attached to poly-L-lysine-coated glass coverslips and cultured for 2 days in RPMI 1640 medium supplemented with 10% fetal calf serum. The cell-coated coverslips were loaded with fura-2 (21), and single-cell $[Ca^{2+}]_i$ measurements and time-resolved digital image analysis were performed as described before (19, 22). Cells were perfused continuously at about 2 ml/ml with a standard solution containing (in mM): NaCl, 145; KCl, 5; $MgCl_2$, 1; $CaCl_2$, 1; glucose, 10; Na-HEPES, 10, pH 7.4. Test substances were added to the perfusion solution. Temperature was 36°C.

For identification of single cells according to the hormone they store, the coverslips were fixed with 4% formaldehyde at the end of the $[Ca^{2+}]_i$ measurements, and indirect immunofluorescence using antibodies raised against one of the pituitary hormones was performed. The field of interest was located by positioning a cross engraved in the coverslip as in the $[Ca^{2+}]_i$ experiment and the fluorescence image was captured with the image processor. The image was digitalized, stored, and later moved and rotated in the computer as required to match exactly the $[Ca^{2+}]_i$ images of the experiment. This procedure has been described in detail elsewhere (23).

Fura-2/AM was obtained from Molecular Probes (Junction City, Oreg.). Antibodies against rat prolactin (rabbit, #AFP425-10-91), β-TSH (rabbit, #AFP1274789), GH (monkey, #AFP4115), β-FSH (guinea pig, #AFP85GP9691BFSHB), and anti-human ACTH (rabbit, #AFP39013082) were generous gifts from the National Hormone and Pituitary Program, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the National Institute of Child Health and Human Development, and the U.S. Department of Agriculture (Rockville, Md.). Fluorescein-labeled anti rabbit, guinea pig, or monkey IgGs, the hypothalamic releasing hormones (GHRH, TRH, GnRH, and CRH) and the glutamate receptor agonists and antagonists were obtained from Sigma (London, U.K.) and RBI (Natick, Mass). The culture medium and the fetal calf serum were from Gibco (Paisley, Scotland). Other chemicals were either from Sigma or E. Merck (Darmstandt, Germany).

TABLE 1. *Frequency distribution of glutamate receptors in anterior pituitary cells*[a]

Receptor	Frequency (%)
Only NMDA	5.9 ± 3.6
Only QA	2.9 ± 1.4
Only KA	3.2 ± 1.7
NMDA & QA	1.2 ± 1.2
NMDA & KA	9.6 ± 6.4
QA & KA	12.0 ± 6.6
NMDA & QA & KA	32.3 ± 8.0
None	34.4 ± 1.4

[a]Values are mean ± SE of the percentage obtained in 5 independent experiments (272 cells). Sequential stimulation with NMDA, kainate (KA), and quisqualate (QA) was as in Fig. 1. Cells were considered responsive when an increase of $[Ca^{2+}]_i$ larger than 50 nM was produced.

RESULTS

Glutamate (100 μM) elicited an increase in $[Ca^{2+}]_i$ in some AP cells. **Figure 1*A*** illustrates a representative example. Half-maximal effect was obtained at about 20 μM glutamate (results not shown). The percentage of cells responding to glutamate ranged between 18 and 94% in 32 experiments with different cell lots (mean±SE, 43±4; 2203 single cells analyzed). We have shown previously that cloned GH_3 pituitary cells responded to glutamate but not to selective glutamate receptor agonists (19). On the contrary, the cell shown in Fig. 1 responded to the glutamate receptor agonists *N*-methyl-D-aspartate (NMDA), kainate (KA), and quisqualate (QA). **Table 1** summarizes the responses to these three agonists in 272 single cells studied in 5 different experiments. About one third of the cells did not respond to any of the three agonists.[3] More than 50% of the cells responded to NMDA and/or KA and 33% responded to all three

[3]In some of these cells the presence of AP hormones was demonstrated by immunocytochemistry (see Table 2). Essentially all the cells in this group possessed voltage-gated Ca^{2+} channels, as they showed a large increase in $[Ca^{2+}]_i$ when they were depolarized with high-K^+ solution. In seven different experiments, the cells responding to depolarization with high-K^+ solution ranged between 91 and 98% of all the cells present in the microscope field.

TABLE 2. *Response to glutamate in the five AP cell types, defined by immunocytochemical identification of the hormone they store*[a]

Stored hormone	% of all the cells	% Responding to glutamate	% Responding to TRH	(Number of experiments/cells)
PRL	58 ± 4	36 ± 17	66 ± 5	(3/264)
GH	40 ± 7	50 ± 5	57 ± 10	(4/333)
TSH	2 ± 1	80 ± 13	87 ± 8	(6/492)
ACTH	18 ± 2	58 ± 10	63 ± 4	(6/477)
FSH	8 ± 1	47 ± 21	61 ± 18	(3/279)

[a]In each experiment the cells were stained, after the $[Ca^{2+}]_i$ measurements, with one of the antibodies against AP hormones, so the existence of cells containing more than one hormone cannot be excluded. Figures are mean ± SE of all the experiments with each antibody (number in the last column).

agonists. The frequencies for the different combinations of glutamate receptors are detailed in Table 1.

The NMDA-induced increase of $[Ca^{2+}]_i$ was fully blocked by 1 μM MK-801, an antagonist of the NMDA receptor (24, 25; results not shown). The effects of KA were mimicked by α-amino-3-hydroxy-5-methyloxazole-4-propionic acid (AMPA, 10 μM) and inhibited by 6,7-dinitroquinoxaline-2,3-dione (DNQX, 8 μM), a specific inhibitor for the kainate/AMPA glutamate receptor (24, 26; results not shown). The increase of $[Ca^{2+}]_i$ induced by KA was fully blocked by 0.1 μM nimodipine (95±2%, mean+SE in 47 single cells; in the same cells nimodipine blocked by 93±8% the increase of $[Ca^{2+}]_i$ induced by depolarization by high-K^+). In contrast, blocking by nimodipine of the increase of $[Ca^{2+}]_i$ induced by NMDA was incomplete (42±14%; $n=40$). Addition of 10 μM glycine together with NMDA increased two- to fourfold the peak $[Ca^{2+}]_i$ attained, whereas it did not modify the increase of $[Ca^{2+}]_i$ induced by KA. Removal of external Ca^{2+} prevented the effects of both NMDA and KA, but had variable effects on the action of QA (results not shown). Quisqualic acid may activate both the kainate/AMPA and the metabotropic class of glutamate receptors (24). Trans-1-amino-1,3-cyclopentanedicarboxylic acid (trans-ACPD, 80 μM), a specific agonist of the metabotropic glutamate receptor (27), elicited a $[Ca^{2+}]_i$ increase (mean±SE) in 13±6% of the cells (378 cells from 7 experiments). Thus, it seems clear that some AP cells do possess functional glutamate receptors.

To ascertain the possible physiological role of glutamate receptors it is pertinent to determine what type or types of AP cells possess glutamate receptors. For these purposes AP cells were stimulated with 100 μM glutamate and then immunocytochemical identification of the cells according to the content of a given AP hormone was carried out in the same microscope field. **Table 2** summarizes the results obtained in 22 different experiments (1845 cells). Cells within all the five cell types responded to glutamate. Although the fraction of responding cells was different in every group (range, 36 to 80%), there was not a clear-cut association between glutamate receptors and a given cell kind. Table 2 also shows that a large proportion of cells (57 to 87%) within each cell type was able to respond to TRH with an increase of $[Ca^{2+}]_i$. This is, to our knowledge, the first demonstration of such a general distribution of TRH receptors within all the AP cell types.

An alternative classification of AP cells was attempted according to their responses to HRHs. All the HRHs tested (GHRH, GnRH, CRH, and TRH) elicited an increase of $[Ca^{2+}]_i$ in a given subpopulation of cells. The left panels in **Fig. 2** illustrate the effects of the four HRHs, perfused sequentially, in five representative single cells. Cells A–C were sensitive to only one HRH (GnRH, GHRH, and TRH, respectively).[4] However, some cells were sensitive to more than one HRH. Cell D was sensitive to both GHRH and TRH. Other cells were sensitive to several combinations of two and three different HRHs (results not shown), and some cells were even sensitive to all four of the HRHs tested (cell E).

In 591 cells studied in 11 experiments, the percentages of cells sensitive to TRH, GHRH, CRH and GnRH were 48, 37, 20, and 15%, respectively. **Figure 3** summarizes the frequency distributions of the different combinations of HRH receptors. About one-third of the cells were not sensitive to any of the HRHs tested (open bar)[3], a second third were sensitive to only one HRH (dashed bars), and the last third were sensitive to more than one releasing hormone (filled bars). About 20% of the cells were sensitive to two different HRHs, 7% were sensitive to three, and another 7% were sensitive to the four HRHs. The most frequent type of cells responsive to multiple HRHs was the one sensitive to TRH and GHRH, followed by the one sensitive to all four of the HRHs. Other combinations were less frequent and some did not exit at all. Particularly, the TRH receptor was always present in the cells responsive to multiple HRHs (Fig. 3). The distribution of cell kinds varied somewhat with the cell batch. For example, the cell subpopulation sensitive to all four HRHs (7% in average) ranged from 4 to 18% in 11 different experiments.

To study the distribution of glutamate receptors in the different cell types defined (Fig. 3), sequential stimula-

[4]The pattern of response to different HRHs was not dependent on the sequence in which they were perfused. This was checked in control experiments in which the four HRHs were applied in different orders to the same microscopic field.

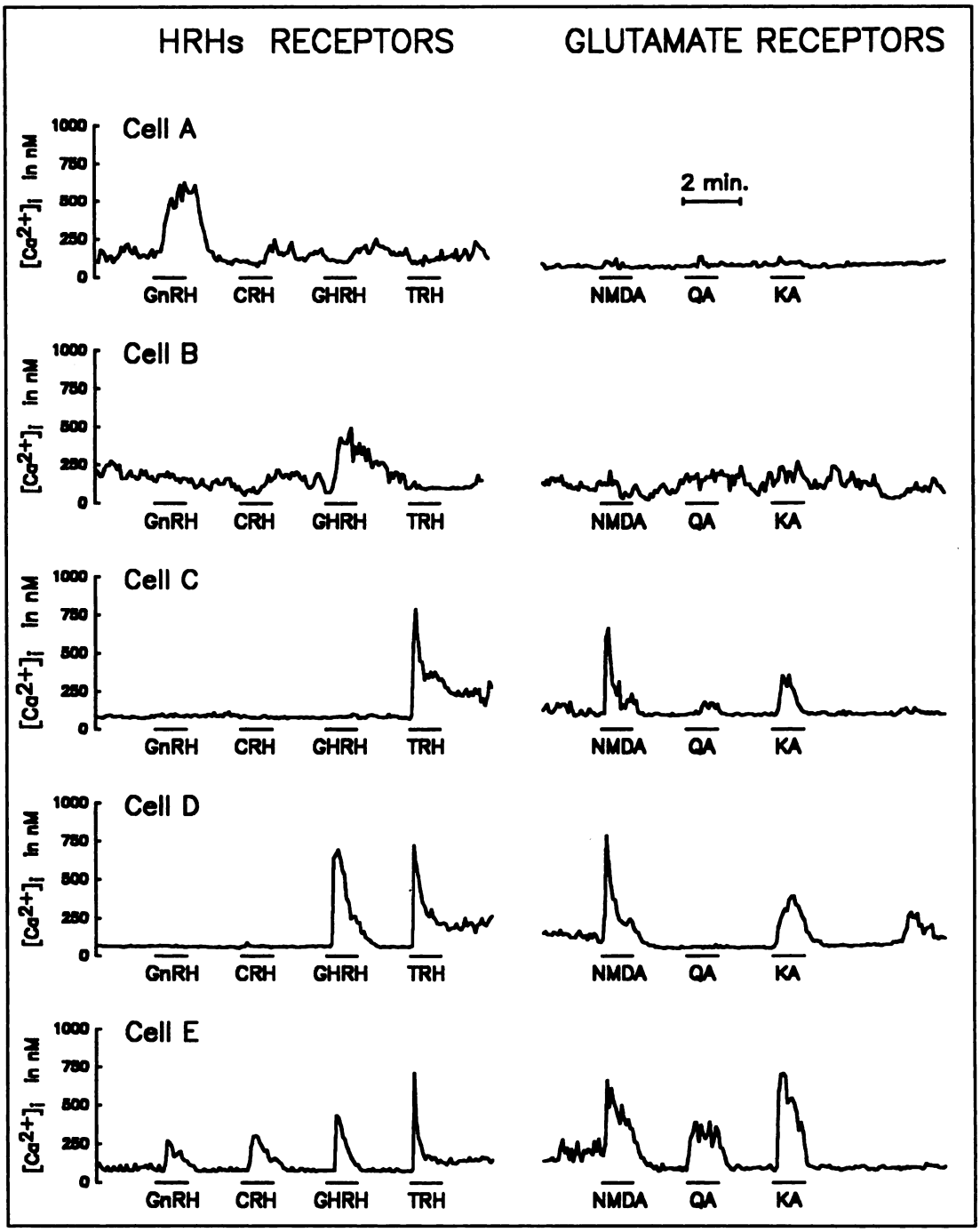

Figure 2. Effects of sequentially perfused hypothalamic releasing hormones and glutamate receptor agonists in five representative single cells. HRHs were tested all at 10 nM except for TRH (100 nM). Concentrations of NMDA, KA, and QA, as in Fig. 1.

tion with the four HRHs and the three glutamate receptor agonists was performed. The results in five representative single cells are shown in the right panels of Fig. 2. Cells A and B, which were sensitive only to GnRH and GHRH, respectively, did not respond to glutamate receptor agonists. In contrast, cells C, D, and E, which were sensitive only to TRH, GHRH and TRH, and all four of the HRHs, respectively, were sensitive to one or several glutamate receptor agonists. What cells C, D, and E have in common is their sensitivity to TRH. When all the cells present in the same microscope field were divided into two groups (the TRH-sensitive and the TRH-insensitive one) and the responses to the glutamate receptor agonists were averaged for each group, a surprising result arose. The responses to the glutamate receptor agonists were restricted to the TRH-sensitive group (**Fig. 4**). Thus, expression of functional TRH and glutamate receptors is closely associated and takes place in the same cells.

Full analysis of the responses to the glutamate receptor agonists in terms of the cell subpopulations defined by

the different combinations of HRHs receptors (Fig. 3) allowed some additional conclusions. Such analysis is summarized in **Fig. 5** for the seven more frequent subpopulations and the glutamate receptor agonist NMDA. Among the cells expressing only one HRH receptor, only those bearing the TRH one coexpressed the glutamate receptor. In cells expressing two HRH receptors (illustrated by the TRH + GHRH combination in Fig. 5), the response to NMDA was significantly greater. Finally, in cells expressing three (not shown in Fig. 5) and the four HRH receptors ("ALL" in Fig. 5), the response to NMDA was still greater. Thus, it seems that, superimposed to the absolute requirement of the presence of the TRH receptor, the expression of functional NMDA receptors is increased in the cells that bear additional HRH receptors. The results with the other glutamate receptor agonists were less clear, although coexpression with the TRH receptor was also observed (Fig. 4). If glutamate receptors are expressed in all the AP cell types and there is an association between glutamate and TRH receptors, it should be expected that TRH receptors are expressed in all the cell types. Confirmation of this expectation has already been shown in Table 2.

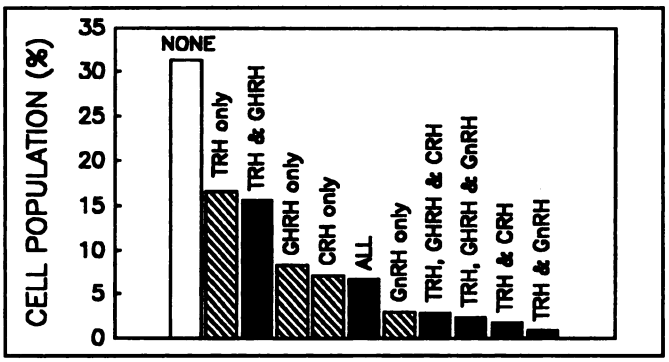

Figure 3. Classification of anterior pituitary cells by their response to the hypothalamic releasing hormones. HRHs were tested at 10 nM except for TRH (100 nM). Frequencies of cells responding to different combinations of HRHs were estimated from the analysis of 591 cells coming from 11 different experiments in which the four HRHs were perfused sequentially, as in Fig. 2. An increase in $[Ca^{2+}]_i$ > 50 nM was considered a positive response. The cells responding to none of the HRHs were, however, responsive to depolarization with high-K^+ solution (see footnote 3).

DISCUSSION

Glutamate has been reported to induce secretion of several AP hormones both in vivo and in vitro (7–13), suggesting that it could act not only at the hypothalamus but also directly on the own AP cells. NMDA has been shown recently to increase $[Ca^{2+}]_i$ in isolated somatotrophs (11). We extend this concept by showing that glutamate is able to increase $[Ca^{2+}]_i$ in all the five types of AP cells, defined by the hormones they store. Both ionotropic (NMDA and kainate/AMPA) and, to a smaller extent, metabotropic glutamate receptors are present.[5] Thus, glutamate could act nonsynaptically (AP is considered a noninnervated tissue) as a releasing factor of pituitary hormones. Nonsynaptic inhibitory regulation of AP secretion has been proposed also for other neurotransmitters such as dopamine or γ-aminobutyric acid (1, 29). On the other hand, the presence of glutamate receptors in pancreatic β cells and a possible role in the control of insulin secretion has been proposed recently (30).

Regarding the origin of glutamate, the most obvious would be secretion from hypothalamic neurons, where glutamate is the dominant excitatory neurotransmitter (9, 31), reaching AP through the portal hypophyseal system. Possible paracrine effects of glutamate released from posterior pituitary axons or pituicytes (15), reaching AP through short portal vessels, should also be considered. Finally, plasma glutamate, which could change depending on food intake and/or the metabolic status, should be taken into account. The resting plasma level of glutamate (40–100 μM) would be enough to produce near-maximal stimulation of AP cells. The activity of AP cells in the intact animal results from the balance of many stimulating and inhibiting factors. Stimulation by plasma glutamate might contribute to provide a basal tone of activity in AP cells, thus leaving room for the action of inhibiting factors such as dopamine or somatostatin. Alternatively, the concentration of glutamate in the plasma reaching AP cells through its portal circulation could be lower than the measured in blood samples. Glutamate could, for ex-

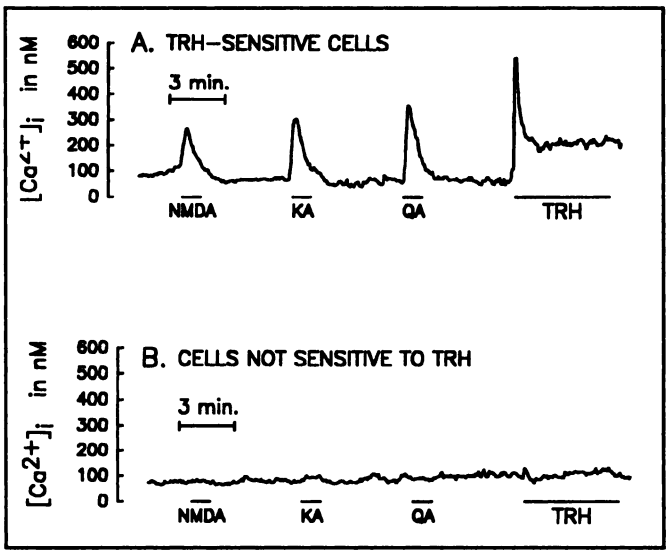

Figure 4. Glutamate receptors are restricted to TRH-sensitive cells. The cells were sequentially perfused with NMDA, KA, QA, and TRH as in Fig. 3. Then the traces for all the cells sensitive to TRH (*A*, 24 cells) or those insensitive to TRH (*B*, 19 cells) were averaged. Results are representative of 12 similar experiments.

[5]This is at variance with previous results in GH_3 cells, where glutamate receptors were absent or nonfunctional (19). Dopamine receptors, which are present in normal lactotrophs and somatotrophs, are also absent in GH_3 cells (28). We also find that GH_3 cells do not respond to angiotensin II whereas normal AP cells do (unpublished observations).

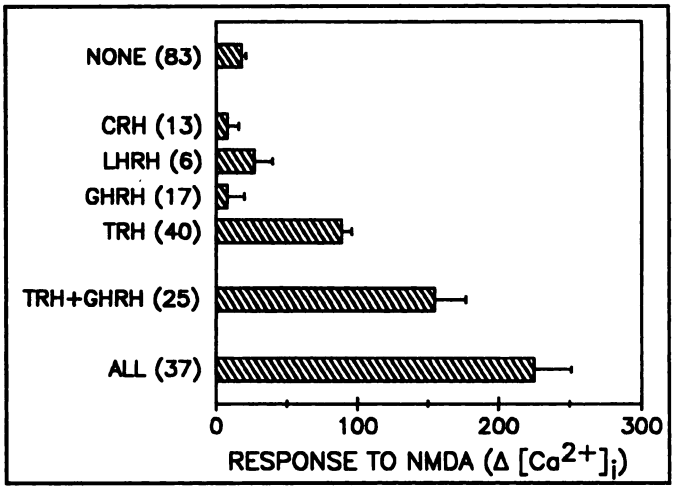

Figure 5. Distribution of NMDA receptors in pituitary cell subpopulations. Cells were sequentially perfused with the HRHs, followed by NMDA, as in Fig. 3. Then they were classified according to their response to the different HRHs, as in Fig. 2. The response to NMDA was quantified in each single cell as the average of the $[Ca^{2+}]_i$ increase during the 1-min period of stimulation. The dashed bars are the means of all the cells studied for each type (number in brackets) in three different experiments. Lines at the right of each bar represent SE. NONE stands for cells not responding to any of the HRHs and ALL for cells responding to all the four HRHs.

ample, be taken up from the capillaries by nearby glial cells during blood circulation through the hypothalamus. In this case, an increase in blood glutamate might be relevant to stimulate AP hormone secretion.

There was no clear-cut association between the cell type, defined by the storage of a given hormone, and the presence or the absence of glutamate receptors. We have attempted an alternative classification of the cells on the basis of the responses to the HRHs. We find that up to one-third of the cells responded to several HRHs, often to all of the four tested. Similar findings have been recently reported by Kasahara et al. (6), although a full population study was not performed in that case. Glutamate and TRH receptors tended to coexpress in the cell kind responsive to multiple HRHs. Because glutamate and TRH are both known to influence cell differentiation (2, 32, 33), it is tempting to speculate that cells responsible to multiple HRHs could play a role in the widely acknowledged phenotypic plasticity of the pituitary gland. Transdifferentiation from one cell kind to another, which is known to happen under the proper humoral influence (34), might include passage through the cell subpopulation sensitive to multiple HRHs.[6] Although our results provide some exciting clues on a possible role of glutamate in a nonneural tissue, further effort, much of it in the intact animal, will be required to elucidate the involvement of glutamate in physiological control of hormone secretion and cell differentiation at the anterior pituitary. FJ

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[6]Some observations in the intact animal are consistent with a role of glutamate in pituitary differentiation and long-term control of hormone secretion (9). For example, glutamate is known to advance the onset of puberty in the rat, whereas glutamate receptor antagonists have the opposite effect (35). Kainate is able to induce LH and FSH secretion in rat AP cells, but sensitivity to kainate is dependent on age, being lost in the older animals (13).

REFERENCES

1. Schally, A. V., Coy, D. H., and Meyers, C. A. (1978) Hypothalamic regulatory hormones. *Annu. Rev. Biochem.* **47**, 89–128
2. O'Leary, R., and O'Connor, B. (1995) Thyrotropin-releasing hormone. *J. Neurochem.* **65**, 953–963
3. Gershengorn, M. C. (1986) Mechanisms of thyrotropin-releasing hormone stimulation of pituitary hormone secretion. *Annu. Rev. Physiol.* **48**, 515–526
4. Kato, M., Hoyland, J., Sikdar, S. K., and Mason, W. T. (1992) Imaging of intracellular calcium in rat anterior pituitary cells in response to growth hormone releasing factor. *J. Physiol. (London)* **447**, 171–189
5. Prevarskaya, N., Skryma, R., Vacher, P., Bresson-Bepoldin, L., Odessa, M. F., Rivel, J., San Galli, F., Guerin, J., and Dufy-Barbe, L. (1994) Gonadotropin-releasing hormone induced Ca^{2+} influx in nonsecreting pituitary adenoma cells: role of voltage-dependent Ca^{2+} channels and protein kinase C. *Mol. Cell. Neurosci.* **5**, 699–708
6. Kasahara, K., Tasaka, K., Masumoto, N., Mizuki, J., Tahara, M., Miyake, A., and Tanizawa, O. (1994) Characterization of rat pituitary cells by their responses to hypothalamic releasing hormones. *Biochem. Biophys. Res. Commun.* **199**, 1436–1441
7. Terry, L. C., Epelbaum, J., and Martin, J. B. (1981) Monosodium glutamate: Acute and chronic effects on rhythmic growth hormone and prolactin secretion, and somatostatin in the undisturbed male rat. *Brain Res.* **217**, 129–142
8. Mason, G. A., Bissette, G., and Nemeroff, C. B. (1983) Effects of excitotoxic amino acids on pituitary hormone secretion in the rat. *Brain Res.* **289**, 366–369
9. Brann, D. W. (1995) Glutamate: A major excitatory transmitter in neuroendocrine regultation. *Neuroendocrinology* **61**, 213–225
10. Login, I. S. (1990) Direct stimulation of pituitary prolactin release by glutamate. *Life Sci.* **47**, 2269–2275
11. Lindström, P., and Ohlsson, L. (1992) Effect of *N*-methyl-D,L-aspartate on isolated rat somatotrophs. *Endocrinology* **131**, 1903–1907
12. Niimi, M., Sato, M., Murao, K, Takahara, J., and Kawanishi, K. (1994) Effect of excitatory amino acid receptor agonists on secretion of growth hormone as assessed by the reverse hemolytic plaque assay. *Neuroendocrinology* **60**, 173–178
13. Zanisi, M., Galliati, M., Messi, M., and Martini, L. (1994) The anterior pituitary gland as a possible site of action of kainic acid. *Proc. Soc. Exp. Biol. Med.* **206**, 431–437
14. Kiyama, H., Sato, K., and Tohyama, M. (1993) Characteristic localization of non-NMDA type glutamate receptor subunits in the rat pituitary gland. *Mol. Brain Res.* **19**, 262–268
15. Meeker, R. B., Greenwood, R. S., and Hayward, J. N. (1994) Glutamate receptors in the rat hypothalamus and pituitary. *Endocrinology* **134**, 621–629
16. Schoepp, D., Bockaert, J., and Sladeczek, F. (1990) Pharmacological and functional characteristics of metabotropic excitatory amino acid receptors. *Trends Pharmacol. Sci.* **11**, 508–515
17. Gasic, G. P., and Hollmann, M. (1992) Molecular neurobiology of glutamate receptors. *Annu. Rev. Physiol.* **54**, 507–536
18. Wisden, W., and Seeburg, P. H. (1993) Mammalian ionotropic glutamate receptors. *Curr. Opin. Neurobiol.* **3**, 291–298
19. Villalobos, C., and García-Sancho, J. (1995) Glutamate increases cytosolic calcium in GH_3 pituitary cells acting via a high-affinity glutamate transporter. *FASEB J.* **9**, 815–819
20. Dobson, P. R. M., and Brown, B. L. (1985) The preparation, culture and incubation of rat anterior pituitary cells for static and dynamic studies of secretion. *Methods Enzymol.* **109**, 293–298
21. Grynkiewicz, G., Poenie, M., and Tsien, R. Y. (1985) A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* **260**, 3440–3450
22. López, M. G., García, A. G., Artalejo, A. R., Neher, E., and García-Sancho, J. (1995) Veratridine induces oscillations of cytosolic calcium and membrane potential in bovine chromaffin cells. *J. Physiol. (London)* **482**, 15–27
23. Núñez, L., De la Fuente, M. T., García, A. G., and García-Sancho, J. (1995) "Differential changes of cytosolic calcium in immunocytochemically identified adrenergic and noradrenergic bovine adrenal chromaffin cells stimulated with various secretagoges." *Am. J. Physiol.* In press
24. Watkins, J. C., and Olverman, H. J. (1987) Agonists and antagonists for excitatory amino acid receptors. *Trends Neurosci.* **10**, 265–272
25. Kemp, J. A., Foster, A. C., and Wong, E. H. F. (1987) Non-competitive antagonists of excitatory amino acid receptors. *Trends Neurosci.* **10**, 294–298

26. Honore, T., Davies, S. N., Drejer, J., Fletcher, E. J., Jacobsen, P., Lodge, D., and Nielsen, F. E. (1988) Quinoxalinediones: potent competitive non-NMDA glutamate receptor antagonists. *Science* **241**, 701–703
27. Palmer, E., Monaghan, D. T., and Cotman, C. W. (1989) Trans-ACPD, a selective agonist of the phosphoinositide coupled excitatory amino acid receptor. *Eur. J. Pharmacol.* **166**, 585–587
28. Cronin, M. J., Faure, N., Martial, J. A., and Weiner, R. I. (1980) Absence of high-affinity dopamine receptors in GH_3 cells: a prolactin-secreting clone resistant to the inhibitory actions of dopamine. *Endocrinology* **106**, 718–723
29. Lamberts, S. W., and McLeod, R. M. (1990) Regulation of prolactin secretion at the level of the lactotroph. *Physiol. Rev.* **70**, 279–318
30. Inagaki, N., Kuromi, H., Gonoi, T., Okamoto, Y., Ishada, H., Seino, Y., Kaneko, T., Iwanaga, T., and Seino, S. (1995) Expression and role of ionotropic glutamate receptors in pancreatic islet cells. *FASEB J.* **9**, 686–691
31. Van den Pol, A. Wuarin, J.-P., and Dudek, F. E. (1990) glutamate, the dominant excitatory transmitter in neuroendocrine regulation. *Science* **250**, 1276–1278
32. Lauder, J. M. (1993) Neurotrasmitters as growth regulatory signals of receptors and second messengers. *Trends Neurosci.* **16**, 233–240
33. Heritier, A. G., and Dubois, P. M. (1993) Influence of thyroliberin on the rat pituitary cell type differentiation: an in vitro study. *Endocrinology* **132**, 634–639
34. Frawley, L. S., and Boockfor, F. R. (1991) Mammosomatotropes: presence and functions in normal and neoplastic pituitary tissue. *Endocrine Rev.* **12**, 337–355
35. Donoso, A. O., Seltzer, A. M., Navarro, C. E., Cabrera, R. J., López, F. J., and Negrovilar, A. (1994) Regulation of luteinizing hormone-releasing hormone and luteinizing hormone secretion by hypothalamic amino acids. *Braz. J. Med. Biol. Res.* **27**, 921–932

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ERRATA

Because of a programming error, superscripts were dropped from several lines at the end of column two, page 182, of E. N. Moudrianakis' article [*FASEB J.* (1996) Protein Coagulation and Reversible Denaturation to the Protein Folding Problem: Chris Anfinsen Defining the Transition, 10, 179–183]. The sentences should have read: We can determine, for example, that the DNA in a single haploid cell (of Lilium) is approximately 53×10^{-12} grams. If we assume that this DNA is present as a single, long double helix, it can be calculated that the length of this coil would be 15×10^7 microns or 15 meters, and that the coiled structure would make 4.4×10^9 turns around the screw axis.

In the letter by D. M. Stearns and K. E. Wetterhahn [*FASEB J.* (1996) Chromium(III) picolinate: authors' reply, **10**, 367–369), the authors were responding to Mr. McCarty's original letter, which assigned reference numbers to personal communications, an unpublished manuscript, and a meeting abstract. Upon typesetting, McCarty's references 3, 4, 23, 28, and 36 were moved to the text and the remaining references were renumbered, but the citations of those references were not changed in the authors' response letter. The following table lists the original numbers as cited in the authors' response, and the corrected numbers cited in McCarty's letter as actually published.

McCarty's original reference numbers as cited in the authors' response (pp 367-369)	Numbers corresponding to McCarty's published references (pp 366-367)
15	13
11	9
5–14, 16–20	3–12, 14–18
25	22
22	20
31	27
33–35	29–31
24	21
37	32

A printing error in the February 1996 issue caused much of figure 2 on page 354 to be obliterated [Van Dam, A.-M., De Vries, H. E., Kuiper, J. Zijlstra, F. J., De Boer, A. G., Tilders, F. J. H., and Berkenbosch, F. (1996) Interleukin-1 receptors on rat brain endothelial cells: a role in neuroimmune interactions? *FASEB J.* **10**, 351–356]. The figure as it should have appeared is printed below.