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Sulfonylurea Receptor mRNA Expression in Pituitary Macroadenomas

Zhuochen Zhu,[1] Ian E. McCutcheon,[2] M. Beatriz S. Lopes,[4] Edward R. Laws, Jr.,[5] Virginia L. Wagner,[5] Janet M. Bruner,[3] Gregory N. Fuller,[3] Lauren A. Langford,[3] Le W. Ang,[1] and Keith E. Friend[1]

[1]Section of Endocrine Neoplasia and Hormonal Disorders; [2]Department of Neurosurgery; and [3]Section of Neuropathology, U.T.M.D. Anderson Cancer Center, Houston, TX; and [4]Departments of Pathology and [5]Neurological Surgery, University of Virginia Health Sciences Center, Charlottesville, VA

ATP-sensitive K^+ (K_{ATP}) channels modulated by sulfonylurea compounds have been previously identified in the anterior pituitary of the rat and have been demonstrated to influence GH release. Recently, a sulfonylurea receptor (SUR) has been cloned from an islet cell tumor and identified as a member of the ATP binding cassette superfamily capable of coupling with inwardly rectifying potassium channels. To determine if the same receptor is expressed in pituitary tumors, SUR mRNA levels were measured in 28 human macroadenoma specimens using an RNase protection assay. All immunonegative, corticotrophin (ACTH), growth hormone (GH), and GH/prolactin (GH/Prl) immunostaining tumors expressed detectable amounts of SUR message. Among these tumors, only the GH and GH/Prl adenomas were functional. Of the tumors immunostaining for luteinizing hormone (LH), follicle-stimulating hormone (FSH), or both, SUR mRNA was present in small amounts in 5/11. Only 1/3 Prl immunostaining tumors contained SUR mRNA. In summary, we have demonstrated that SUR mRNA expression is common in several types of silent pituitary adenomas and in functional tumors that secrete GH. Lower levels are seen in some gonadotrophin immunostaining tumors.

Key Words: Pituitary gland; sulfonylurea receptor; pituitary adenomas.

Introduction

The recently cloned sulfonylurea receptor (SUR) is an ATP-binding cassette containing protein that appears to interact with at least one member of the inward rectifier K^+ channel family to confer ATP-sensitivity on K^+ channel function (K_{ATP}) *(1–3)*. Mutations in the SUR are associated with familial persistent hyperinsulinemic hypoglycemia *(4)*. K_{ATP} channels are an important regulator of glucose-induced insulin secretion in the pancreas and have also been demonstrated to modulate growth hormone (GH) secretion in the rat *(5,6)*. With regard to GH secretion, [^{3}H] glibenclamide binding sites, which are generally considered reliable markers for K_{ATP} channels, are widely distributed throughout the rat anterior pituitary, and although two to three times less abundant than in the pancreatic β-cell, they exhibit similar absolute and rank order of affinities to other sulfonylureas *(5)*. In addition, it has been demonstrated in primary cultures that both glucose and sulfonylurea drugs depolarize rat anterior pituitary cells, whereas diazoxide induces a hyperpolarization. Accordingly, diazoxide inhibits GH release in a manner that is antagonized by sulfonylureas *(5)*. In a similar experimental system, it has also been demonstrated that sulfonylureas antagonize somatostatin inhibition of GH secretion, whereas diazoxide antagonizes growth hormone-releasing hormone (GHRH) factor-stimulated release *(6)*, indicating that K_{ATP} channels may also be important in modulating somatotrope function in response to hypothalamic factors. The molecular weight of the pituitary SUR is 140–150 kDa, similar to that seen in the pancreas *(5)*. Indeed, SUR mRNA expression has been recently demonstrated in normal rat pituitary and in GH3 cells, a GH and prolactin (Prl) producing rat anterior pituitary cell line *(7–9)*.

The GH response in humans to feeding is usually one of suppression, whereas hypoglycemia generally increases GH release. This is in marked contrast to what would be predicted if the SUR were important in directly modulating somatotrope function in a manner similar to that of the pancreatic β-cell or the rat anterior pituitary. In acromegaly, however, glucose fails to suppress GH levels and induces a paradoxical GH increase in approx 15% *(10)*. This could occur if SUR expression is upregulated in GH-

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Author to whom all correspondence and reprint requests should be addressed: K. E. Friend, MD, U. T. M. D. Anderson, Box 15, 1515 Holcombe Boulevard, Houston, TX 77030. E-mail: Kfriend@endocrine.mdacc.tmc.edu

producing tumors or if it is no longer subservient to hypothalamic regulation. To determine if the SUR is indeed expressed in human pituitary adenomas, SUR mRNA levels were measured in 28 human macroadenoma specimens using an RNase protection assay (RPA); SUR mRNA expression was detectable in 20 specimens, with the highest levels occurring in corticotrophin (ACTH), GH, and immunonegative tumors; normal pituitary gland also contained SUR mRNA. The presence of SUR in both normal and adenomatous tissue suggests that it may influence hormone release in the pituitary as well as the pancreas.

Results

Analysis of Pituitary Adenomas for SUR mRNA

A total of 28 pituitary adenomas were analyzed for SUR mRNA using an antisense RNA probe complementary to the NBF-2 region of the SUR (Fig. 1). Of these, 20 tumor specimens contained detectable amounts of SUR message. The results of RPA for SUR are shown in Fig. 2. As demonstrated, a human β-actin RNA probe was used as an internal control for each sample. Although the specific activity of the β-actin probe was reduced by 100-fold in relation to the SUR probe by adding increased unlabeled nucleotide in transcribing the β-actin template, as described in the Materials and Methods section, in most instances the β-actin signal is still significantly higher than that of SUR. This indicates that the relative abundance of SUR mRNA is, as expected, significantly less than β-actin mRNA. Normal pituitary gland obtained at autopsy—pooled from four specimens obtained from individuals of various ages and both genders—contained small but detectable amounts of SUR mRNA (SUR mRNA: β-actin mRNA, 0.05). Because RNase protection is quite sensitive in detecting the presence of target message, each specimen in this study was also examined for the presence of thyroid stumulating hormone (TSHβ) mRNA as a marker for contamination with normal pituitary tissue. This methodology has been previously employed *(11,12)*. TSHβ was chosen because it is produced by only 1–3% of pituitary adenomas *(13)*. As demonstrated in Fig. 3, TSHβ mRNA was abundant in normal pituitary gland obtained at autopsy but was absent in the adenoma specimens.

Relationship of Tumor Immunohistochemistry to SUR mRNA

SUR mRNA RPA data and tumor immunohistochemical staining results are crossreferenced in Table 1. All immunonegative tumors expressed SUR mRNA, and this subset of tumors possessed the highest absolute levels of SUR message relative to the β-actin mRNA internal control. All ACTH, GH, and GH/Prl immunostaining tumors also expressed detectable amounts of SUR message, with the mean relative amount being somewhat less, but not statistically different from that seen in the immunonegative tumors. All ACTH immunostaining tumors were clinically silent in that there was no clinical or biochemical evidence of cortisol excess. With regard to those tumors immunostaining for either luteinizing hormone (LH), follicle-stimulating hormone (FSH), or both, SUR mRNA was present in 5/11. Tumors that immunostained for LH or FSH were broken down into two categories based on their appearance on electron microscopy (EM). Those with the appearance of gonadotrope cells (elongated cells with well-developed rough endoplasmic reticulum and Golgi) were separated from those with a null cell appearance (polygonal cells with an irregular nucleus and poorly developed rough endoplasmic reticulum and Golgi complex) as has been suggested by Horvath and Kovacs *(14)*. Although the null cell tumors are generally less efficient at producing hormone than the gonadotrope-like tumors, both will react with gonadotrophin antisera. For this reason, the distinction between the null cell and gonadotrope tumors needs to be made at the ultrastructural level. Within the gonadotrophin immunostaining group, tumors exhibiting a gonadotrope appearance on EM were more likely to express SUR message (5/6) than those that those that had a null cell or oncocytic (null cell with elevated mitochondrial content) appearance (0/5). Only 1/3 Prl immunostaining tumors contained SUR mRNA, and the absolute amount in this tumor was relatively low. No adenomas with positive immunoreactivity for TSH were included in the study.

Clinical data on the patients with GH, GH/Prl, Prl, and ACTH immunostaining tumors are shown in Table 2. As indicated, these tumor specimens were obtained from a variety of patients with regard to age and clinical presentation. The patients with ACTH immunostaining tumors had no evidence of cortisol excess clinically or biochemically. None of the patients with gonadotrophin or immunonegative tumors presented with symptoms of a hormonal excess syndrome, and all came to clinical attention because of mass lesion effects, such as development of visual field abnormalities.

Discussion

Our experiments demonstrate that SUR mRNA is expressed in both the normal pituitary gland and in several types of pituitary adenomas. The highest levels were seen in immunonegative as well as ACTH and GH immunostaining tumors. Of these, it is important to note that only the GH adenomas were functional. Therefore, this would be the only subset of adenomas that would be likely to demonstrate a measurable secretory response to factors modulating SUR function. Because this series was limited to macroadenomas in order to eliminate contamination with any normal pituitary tissue, no ACTH-secreting tumors (predominately microdenomas) were available to be studied. All those included in this study were silent. The biochemical significance of SUR expression in nonreactive tumors but not in LH/FSH immunostaining tumors with

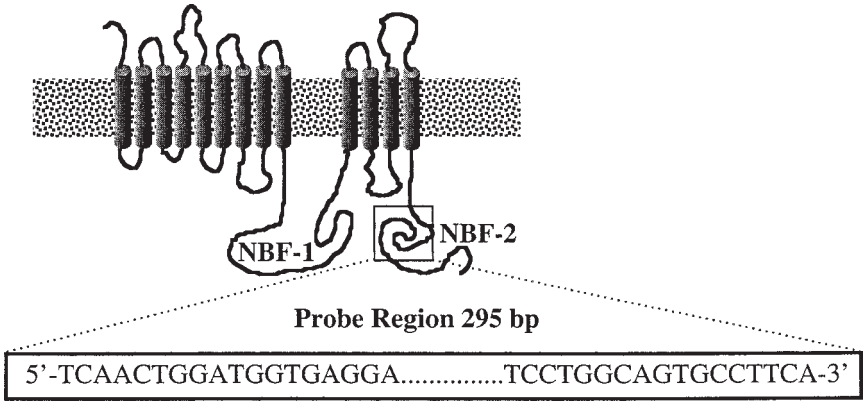

Fig. 1. Schematic diagram of the SUR and location of the RNA probe used for RPA.

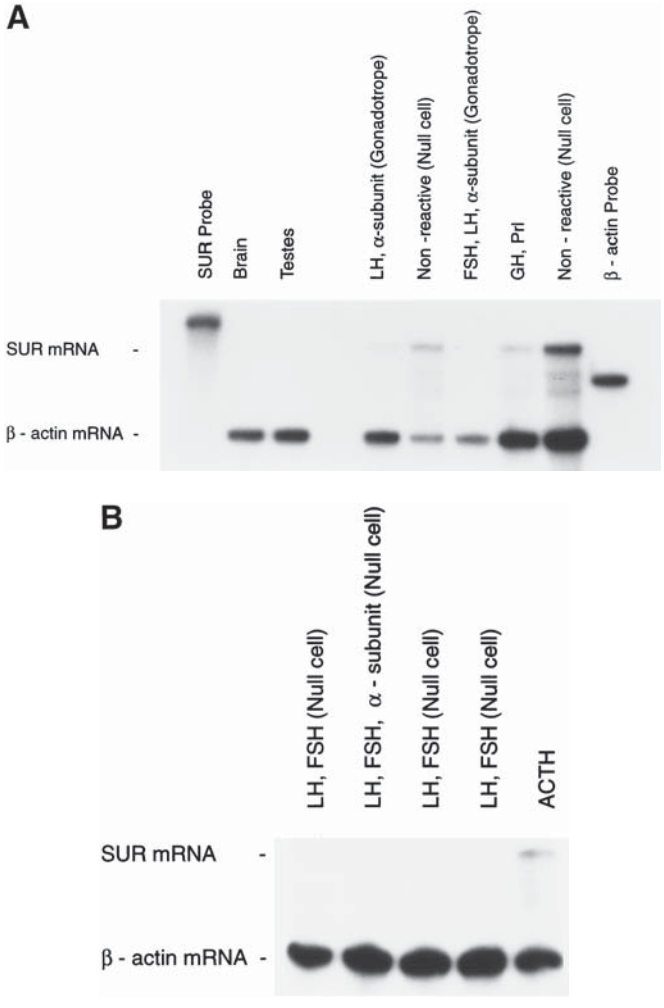

Fig. 2. RPA using SUR and β-actin probes. The position of protected bands for each probe is shown. Twenty micrograms of total RNA from each normal tissue and tumor were used in the assay. Immunostaining results for each tumor are shown above each lane. **(A)** Undigested SUR and β-actin probes, RNA isolated from normal testes, brain, and pituitary adenoma specimens. **(B)** Pituitary adenoma specimens.

null cell EM characteristics is not readily apparent and may simply reflect differences in cell of origin.

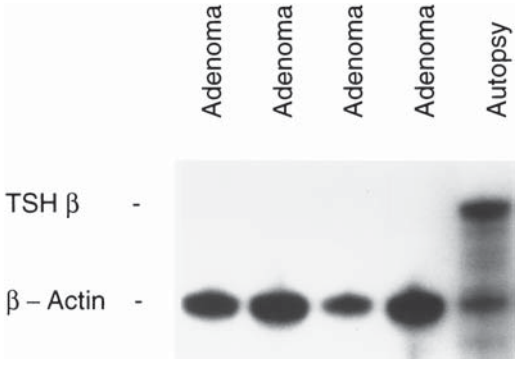

Fig. 3. RPA using TSHβ probe. The position of protected bands for each probe is shown. Ten micrograms of total RNA from each tumor were used in the assay. The autopsy specimen was used as a positive control.

Table 1
SUR RPA, Tumor Immunostaining, and EM Results

Immunohisto-chemistry	Electron microscopy	SUR (+)/ tested	Mean SUR mRNA + S.E.
Nonreactive	Null cell	3/3	0.58 ± 0.07[b]
ACTH		3/3	0.23 ± 0.11[b,c]
GH		3/3	0.17 ± 0.07[b,c,d]
GH/PRL		5/5	0.15 ± 0.02[b,c,d]
LH/FSh	Gonadotroph	5/6	0.08 ± 0.03[c,d]
PRL		1/3/	0.02 ± 0.02[c,d]
LH/FSH	Null cell	0/5	0.00 ± 0.00[d]

[a]Ratio of SUR to β-actin RNA levels, determined by densitometric measurement of autoradiographs.

[b,c,d]Denote when $P < 0.05$ using Kruskal–Wallis test and Dunn's multiple comparison test.

The presence of SUR mRNA in the human pituitary gland is consistent with several previous observations made in primary culture studies in the rat, namely that K_{ATP} channels can directly modulate GH secretion and that pituitary [^{3}H] glibenclamide binding is substantial *(5,6)*. The RPA experiments performed here have demonstrated not only that SUR mRNA is present in both normal and adenomatous

Table 2
Clinical Data, Immunostaining Results, SUR Analysis

Age/gender	Immuno-staining	Laboratory data	SUR status[a]	Note
41 M	GH	GH 19 μg/L, IGF 1006 ng/mL	0.25	Invasive
25 F	GH	GH 3.3 μg/L, IGF-1 287 ng/mL	0.20	Invasive
39 M	GH	GH 53 μg/L, IGF-1 1029 ng/mL	0.03	Invasive
46 F	GH/Prl	GH N/A, IGF-1 731 ng/mL, Prl 291 μg/L	0.20	Noninvasive
36 M	GH/Prl (weak)	GH 22 μg/L, IGF-1 1500 ng/mL, Prl 4 μg/L	0.15	Noninvasive
37 M	GH/Prl (weak)	GH 11 μg/L, IGF-1 1500 ng/mL, Prl 10 μg/L	0.15	Invasive
23 F	GH/Prl	GH 44 μg/L, IGF-1 940 ng/mL, Prl 22 μg/L	0.15	Noninvasive
33 M	GH/Prl (weak)	GH 12 μg/L, IGF-1 662 ng/mL, Prl 28 μg/L	0.09	Invasive
40 F	Prl	Prl 50 μg/L	0.07	Invasive
40 M	Prl	Prl 3090 μg/L	0.00	Invasive
51 M	Prl	Prl 87 μg/L	0.00	Invasive
53 F	ACTH	Cortisol 8 μg/dL	0.30	Invasive
50 F	ACTH	Cortisol 3 μg/L	0.27	Invasive
46 M	ACTH	Cortisol N/A	0.08	Invasive

[a]Ratio of SUR to mRNA to β-actin mRNA as per Table 1.

human pituitary, but that the pituitary and pancreas both contain similar if not identical transcripts, at least in the NBF-2 region encompassed by our probe. The development of antibodies specific for the SUR is required for the immunocytochemical studies necessary to confirm that protein expression parallels that observed with mRNA.

The precise functional mechanisms by which changes in glucose concentrations alter hormone release are best understood in the pancreatic β-cell *(15)*. Extracellular glucose levels are thought to be transduced into the appropriate insulin secretory response as the result of a series of events beginning with efficient transfer of glucose across the β-cell membrane, such that equilibrium with the intracellular environment is rapidly established *(16)*. Glucose is then phosphorylated primarily by glucokinase to glucose 6-phosphate, the majority of which is metabolized in the glycolytic pathway, resulting in an increased intracellular ATP/ADP ratio. This is thought to be detected by the SUR, an ATP binding cassette containing protein, causing closure of the K^+ channel to which it is coupled, and resulting in plasma membrane depolarization *(17,18)*. This depolarization triggers the gating of voltage-sensitive Ca^{2+} channels producing a rise in intracellular Ca^{2+} that results in insulin secretion, perhaps by stimulating contraction of an intracellular network of actin-like microfilaments *(19)*. The degree to which this model is directly applicable to glucose effects in the somatotrope remains undefined, although the existence of K_{ATP} and voltage-sensitive calcium channels in these and other types of pituitary cells is well known *(20,21)*.

Further information is required about SUR distribution in the hypothalamus, particularly with respect to the arcuate nucleus that contains the majority of GHRH neurons and those regions, such as the periventricular area, that contain significant numbers of tuberoinfundibular somatostatin (SS) neurons. If the SUR is expressed in one of these areas and is an important modulator of GHRH or SS secretion, then direct pituitary effects may only become relevant when a disconnection between the pituitary and hypothalamus occurs or if the primary pituitary tissue escapes normal hypothalamic regulation. The paradoxical rise that occurs in some individuals with acromegaly might represent one such condition. Further study is also needed to determine if the SUR-positive tumors represent a subset of adenomas that have distinct characteristics in terms of response to other secretogogs or pharmacological agents used to treat these tumors, such as has been described for

those tumors harboring somatic mutations in the gene encoding the α-subunit of the heterotrimeric guanosine 5'-triphosphate-binding protein, $G_{s\alpha}$. Tumors with these mutations have been reported to have a greater decrease in GH following a glucose load and to respond better to somatostatin than those without these mutations *(22–24)*.

In summary, we have demonstrated that SUR mRNA is present in normal human pituitary gland and several types of silent pituitary adenomas, as well as functional tumors that secrete GH. In the NBF-2 region encompassed by our probe, pituitary, and pancreas SUR transcripts are similar, if not identical. Because the role of SUR in regulating glucose-mediated insulin secretion in the pancreas is now well-established, the expression of this receptor in the pituitary makes it possible that it is performing a similar function with respect to mediating the effects of glucose on GH secretion. Along with previous physiological studies demonstrating that K_{ATP} channels are an important regulator of glucose-induced GH secretion in the pituitary (SUR appears to be one component of these channels) *(5,6)*, our findings with respect to SUR expression in the pituitary gland will provide a foundation for future physiological and molecular studies to examine the mechanisms of how glucose modulates the secretion of pituitary hormones.

Materials and Methods

RNA Isolation

Immediately after excision, the surgical specimens were divided into two portions; one was sent for routine histopathologic diagnosis including immunohistochemical staining and EM, whereas the other was stored at –80°C for RNA isolation. The specimens were carefully stripped to remove any normal pituitary tissue present in the specimens; for this reason, the study was limited to macroadenomas. The diagnosis of pituitary adenoma was confirmed by routine histopathologic examination in all cases. The tumors analyzed represent a consecutive series of tumor specimens. Autopsy specimens of normal anterior pituitary tissue were obtained from patients 6–12 h after expiration. All tissue specimens were obtained in accordance with the guidelines of the Human Investigation Committees at the University of Virginia and University of Texas, M. D. Anderson Cancer Center.

Total RNA was extracted from pituitary adenomas or normal pituitaries by the cesium chloride ultracentrifugation method *(25)*. Briefly, tumor or autopsy tissue was solubilized in guanidine isothiocyanate buffer using a sonicator; the lysate was layered onto a 5.7 *M* CsCl solution and spun in an ultracentrifuge at 111,000*g* for 24 h. RNA was then recovered from the pellet at the bottom of the tube.

RNase Protection Assay

A cDNA clone containing the nuclear binding fold-2 (NBF-2) region of human SUR was kindly provided by Thomas et al. *(4)*. This cDNA fragment was obtained from human pancreatic tissue using the following two primers in RT-PCR amplification (5'-TCAACTGGATGGTGAGGA-3', 5'-TCCTGGCAGTGCCTTCA-3'). The location of the region of the SUR used as an antisense RNA probe for these studies is depicted in Fig. 1. To produce RNA probe, the plasmids were linearized and transcribed with T7 RNA polymerase in the presence of [α-^{32}P]UTP, 40 m*M* Tris-HCl (pH 7.5), 6 m*M* $MgCl_2$, 2 m*M* spermidine, 10 m*M* NaCl, 10 m*M* ATP, CTP, and GTP, 100 m*M* dithiothreitol; and 20 U RNase inhibitor. A 125-bp fragment RNA probe of the human β-actin gene was produced in a similar manner to be used as an internal control for loading efficiency, and so forth. To maintain autoradiograph exposure in the linear range for both probes and still saturate β-actin target, a high specific activity SUR probe (1×10^9 cpm/μg) was combined with a low specific activity β-actin probe (1×10^7 cpm/μg). As previously described, a 198-bp segment of the human TSHβ cDNA corresponding to the coding region for amino acids 2–67 (26) was used in a separate RNase protection assay as verification that the target sample contained only tumor tissue *(11,12)*. Radiolabeled probes were gel-purified, and 1×10^5 cpm (≈1.2 ng for SUR; 600 pg for TSHβ) were incubated for 18 h with 5–20 μg sample RNA using the buffer system supplied in the RPA II kit (Ambion, Austin, TX). A 1:100 dilution of the RNase A/T1 mixture supplied with the kit was used to digest the hybrids. The resulting products were separated on an 8 m*M* urea–5% acrylamide gel and subsequently exposed to X-ray film with an intensifying screen for 1 wk.

Immunohistochemical Staining

Tumor specimens were fixed in 10% phosphate-buffered formalin, dehydrated in graded alcohols and xylene, and embedded in paraffin. Four- to 5-μm paraffin sections were subsequently mounted on glass slides for light microscopy and immunocytochemistry. As previously described, a modified avidin–biotin–peroxidase technique was used for immunocytochemistry *(27,28)*. Briefly, tissue-mounted slides were deparaffinized and processed to alcohol. To remove endogenous peroxidase activity and block nonspecific antibody adherence, the slides were treated with methanolic hydrogen peroxide and 3% normal serum, respectively. The following primary antisera directed against human (h) GH (1:3000 dilution), hPRL (1:2000), hACTH (1:2000), hLHβ (1:3000), hFSHβ (1:2000), and hTSHβ (1:3000) which were obtained from Dako Corp (Carpinteria, CA) were then applied on the slides. Negative controls in which the primary antibodies were replaced with nonimmune serum were also performed. The avidin–biotin complex technique was used to detect the hormone–antisera hybrids. The immunohistochemistry results were blinded until the SUR mRNA analysis was complete.

Data Analysis

For estimation of SUR mRNA levels, autoradiographs of RPA bands were quantified by scanning densitometry.

mRNA levels were determined by the calculation of the image density of the SUR mRNA band divided by that of the β-actin band. Analysis of variance among pituitary tumor groups was done by Kruskal-Wallis test, and Dunn's multiple comparison test was performed to exam pairwise differences whenever necessary.

Hormonal Measurements

Serum IGF-1 levels (normal range 123–463 mg/mL) were measured by Nichols Institute (San Juan Capistrano, CA). Serum Prl (normal range 0–15 μg/L), GH (normal range 0–5 μg/L), and cortisol were measured using in-house RIAs.

References

1. Aguilar-Bryan, L., Nichols, C. G., Wechsler, SW., Clement, J. P. IV, Boyd, A. E. III, Gonzalez, G., et al. (1995). *Science* **268,** 423–426.
2. Philipson, L. H. and Steiner, D. F. (1995). *Science* **268,** 372–373.
3. Ammala, C., Moorhouse, A., Gribble, F., Ashfield, R., Proks, P., Smith, P. A., et al. (1996). *Nature* **379,** 545–548.
4. Thomas, P. M., Cote, G. J., Wohllk, N., Haddad, B., Mathew, P. M., Rabl, W., et al. (1995). *Science* **268,** 426–429.
5. Bernardi, H., De Weille, JR., Epelbaum, J., Mourre, C., Amoroso, S., Slama, A., et al. (1993). *Proc. Natl. Acad. Sci. USA* **90,** 1340–1344.
6. De Weille, J. R., Fosset, M., Epelbaum, J., and Lazdunski, M. (1992). *Biochem. Biophys. Res. Commun.1***87,**1007–1014.
7. Inagaki, N., Tsuura, Y., Namba, N., Masuda, K., Gonoi, T., Horie, M., et al. (1995). *J. Biol. Chem.* **270,** 5691–5694.
8. Inagaki, N., Gonoi, T., Clement, J. P. IV, Namba, N., Inazawa, J., Gonzalez, G., et al. (1995). *Science* **270,** 1166–1170.
9. Dixon, A. K., Gubitz, A. K., Ashford, M. L., Richardson, P. J., and Freeman, T. C. (1995). *FEBS Lett.* **374,** 135–140.
10. Cryer, P. E., Jacobs, L. S., and Daughaday, W. H. (1973). *Mt. Sinai J. Med.* **40,** 402–408.
11. Friend, K. E., Chiou, Y. K., Laws, E. R. Jr, Lopes, M. B., and Shupnik, M. A. (1993). *J. Clin. Endocrinol. Metab.* **77,** 1281–1286.
12. Friend, K. E., Chiou, Y. K., Lopes, M. B., Laws, E. R. Jr, Hughes, K. M., and Shupnik M. A. (1994). *J. Clin. Endocrinol. Metab.* **78,** 1497–1504.
13. Trouillas, J., Girod, C., Loras, B., Claustrat, B., Sassolas, G., Perrin, G., et al. (1988). *Pathol. Res. Pract.* **183,** 596–600.
14. Horvath, E. and Kovacs, K. (1984). *Am. J. Pathol.* **117,** 429–440.
15. Boyd A. E. III, Aguilar-Bryan, L., Bryan, J., Kunze, D. L., Moss, L., Nelson, D. A., et al. (1991). *Recent Prog. Horm. Res.* **47,** 299–317.
16. Cook, D. L. (1995). *Diabetes Res. Clin. Pract.* **28,** S81–89.
17. Ashcroft, F. M. and Kakei, M. (1989). *J. Physiol.* **416,** 349–367.
18. Panten, U., Schwanstecher, M., and Schwanstecher, C., (1996). *Exp. Clin. Endocrinol.* **104,** 1–9.
19. Boschero, A. C., Bordin, S., Plasman, P. O., and Herchuelz, A. (1992) *Horm. Metab. Res.* **24,** 51–52.
20. Sims, S. M., Lussier, B. T., and Kraicer, J. (1991). *J. Physiol.* **441,** 615–637.
21. Edwards, G. and Weston, A. H. (1993). *Ann. Rev. Pharmacol. Toxicol.* **33,** 597–637.
22. Spada, A., Arosio, M., Bochicchio, D., Bazzoni, N., Vallar, L., Bassetti, M., et al. (1990). *J. Clin. Endocrinol. Metab*. **71,** 1421–1426.
23. Landis, C. A., Harsh, G., Lyons, J., Davis, R. L., McCormick, F., and Bourne, H. R. (1990). *J. Clin. Endocrinol. Metab.* **71,** 1416–1420.
24. Adams, E. F., Brockmeier, S., Friedman, E., Roth, M., Buchfelder, M., and Fahlbush, R. (1993). *Neurosurgery* **33,** 198–203.
25. Chirgwin, J. M., Przybyla, A., MacDonald, R. J., and Rutter, W. J. (1979). *Biochemistry* **18,** 5284–5299.
26. Wondisford, F. E., Radovick, S., Moates, J. M., Usala, S. J., and Weintraub, B. D. (1988). *J. Biol. Chem.* **263,** 12,538–12,542.
27. Heitz, P. U., Landolt, A. M., Zenklusen, H. R., Kasper, M., Reubi, J. C., Oberholzer, M., et al. (1987). *J. Histochem. Cytochem.* **35,** 1005–1011.
28. Hsu, S. M., Raine, L., and Fanger, H. (1981). *Am. J. Clin. Pathol.* **75,** 816–821.