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Ontogenic expression of anterior pituitary $GABA_B$ receptor subunits

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Abstract

γ-Aminobutyric acid (GABA) is involved in the neuroendocrine control of hypophyseal secretion, acting both in the central nervous system and directly at the pituitary. We have characterized the properties of anterior pituitary $GABA_B$ receptors. In this work the ontogeny of rat anterior pituitary $GABA_B$ receptors and the pattern of subunit expression in rats of both sexes were determined. Western blot analysis showed a temporal decrease in $GABA_B$ subunits $GABA_{B(1a)}$ and $GABA_{B(1b)}$ expression in female anterior pituitary membranes from day 4 to adulthood, with $GABA_{B(1a)}$ being significantly more abundant than $GABA_{B(1b)}$ at early stages of development; the $GABA_{B(2)}$ subunit was barely detectable. In the male, $GABA_{B(1a)}$ followed a similar pattern and appeared to be significantly less abundant than in 4- and 12-day-old females; $GABA_{B(1b)}$ and $GABA_{B(2)}$ expression in the male was barely detectable. Scatchard plot analysis showed a temporal decrease in binding sites in female anterior pituitary membranes, in agreement with the western blot results. The number of binding sites was significantly higher in female than in male 4-day-old membranes. Dissociation constant values were similar for both sexes at all ages studied.

This study reports for the first time the ontogeny of anterior pituitary $GABA_B$ receptors, showing a particular developmental pattern of subunit expression and a clear sexual dimorphism. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: $GABA_B$ receptors; Anterior pituitary; Ontogeny

1. Introduction

It has been clearly shown that γ-aminobutyric acid (GABA) is involved in the neuroendocrine control of hypophyseal secretion, acting both in the central nervous system (CNS) and directly at the pituitary. Different neuropharmacological agents, acting at $GABA_A$ and $GABA_B$ receptors, can alter neuroendocrine GABAergic regulation. GABA receptors and biosynthetic enzymes have particular ontogenic distributions in different CNS areas including locations critically involved in the control of pituitary hormone secretion (Duvilanski et al., 1984; Lacau-Mengido et al., 1989). In the rat brain, a switch in the subunit composition of $GABA_A$ receptors during postnatal development has been determined (Fritschy et al., 1994). Furthermore, distinct patterns of pituitary hormone responses to GABA, acting at both $GABA_A$ and $GABA_B$ receptors, have been described in developing male and female rats (Rey-Roldán et al., 1997; Moguilevsky et al., 1992). Pituitary $GABA_A$ binding sites have been described (Grandison, 1981; Fiszer de Plazas et al., 1982). The presence of hypophyseal $GABA_B$ binding sites was strongly suggested by our own “in vitro” hormonal studies (Lux-Lantos et al., 1992; Rey-Roldán et al., 1996) and was demonstrated in the adult male rat in binding studies (Anderson and Mitchell, 1986). In addition, we have recently reported that baclofen action in adenohypophyseal cells is Pertussis toxin sensitive and that the anterior pituitary $GABA_B$ receptor is negatively coupled to adenylyl cyclase and to calcium ion channels (Lux-Lantos et al., 1999), as it

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was described for presynaptic $GABA_B$ binding sites in the CNS (Bowery, 1999).

With regard to their ontogenic distribution, $GABA_B$ binding sites appear to be present in the rodent brain at an early stage of life and peak at regionally specific times during the first three weeks of life and then decline to adult levels (Turgeon and Albin, 1994; Bowery, 1996). Others have reported that receptor protein expression either decreases or does not change along ontogeny, depending on the region of the nervous system analyzed (Malitschek et al., 1998). No data are available about the developmental expression of $GABA_B$ receptors in the anterior pituitary. In recent studies, neuronal $GABA_B$ receptors have been reported to assemble into heterodimers formed by $GABA_B$R1a/b and $GABA_{B(2)}$ subunits (Kaupmann et al. 1997, 1998; Jones et al., 1998; Kuner et al., 1999; White et al., 1998). Besides $GABA_{B(1a)}$ and $GABA_{B(1b)}$, additional splice variants of the $GABA_{B(1)}$ receptor gene have also been identified in various CNS and peripheral tissues (Isomoto et al., 1998; Pfaff et al., 1999; Ekstrand et al., 1999).

Given the importance of GABA for pituitary function and the alterations in this neuroendocrine control occasioned by neuropharmacological drugs acting on GABA receptors, the aim of the present study was to determine the ontogeny of anterior pituitary rat $GABA_B$ receptors in both sexes.

2. Materials and methods

2.1. Animals

Male and female virgin Sprague-Dawley rats (200–250 g) from the Instituto de Biología y Medicina Experimental colony were housed in groups in an air-conditioned room, with lights on from 7.00 a.m. to 7.00 p.m. They were given free access to laboratory chow and tap water. Mothers with their pups were kept undisturbed in individual cages. Pups were allowed to remain with their mothers until the day of the experiment. Day of birth was considered as day 1. Ages studied were 4, 12, 20, 28, 37 days and adults (adult female rats were used in proestrus or at 15 days of lactation). Animals were killed by decapitation, around 9.00 a.m., to avoid circadian variations. All procedures were according to protocols for animal use, as approved by the Institutional Animal Care and Use Committee (IBYME–CONICET) that follows NIH guidelines.

2.2. Membrane preparation

Anterior pituitaries were collected and the membrane fraction was isolated according to the method of Olpe et al. (1990). Briefly, pituitaries were homogenized in 10 volumes of ice-cold 0.32 M sucrose, containing 1 mM $MgCl_2$ and 1 mM K_2HPO_4, with a glass/Teflon homogenizer. Membranes were centrifuged at 750**g**, the pellet was resuspended and the centrifugation repeated. The supernatants were pooled and centrifuged at 18 000**g** for 15 min. The pellet was osmotically shocked, centrifuged at 39 000**g**, resuspended in 50 mM Tris–HCl, 2.5 mM $CaCl_2$, pH 7.4 (10 vol/g of original tissue), and washed twice. Membranes were frozen at −70°C.

2.3. Western blot analysis

Western blot analysis of $GABA_B$ receptor subunits was performed as described by Malitschek et al. (1998). Briefly, 30–50 μg of pituitary membrane preparations were subjected to 14–4% gradient SDS–PAGE. Proteins were transferred onto nitrocellulose by standard wet electrophoretic transfer in a 0.2 M phosphate buffer. Blots were blocked in NETG buffer (150 mM NaCl; 5 mM EDTA; 50 mM Tris–HCl, pH 7.4; 0.05% Triton X-100; 0.25% gelatin) for 30 min with three changes of buffer. $GABA_B$ subunits were detected by incubating for 30 min at room temperature with antibodies Ab174.1 (1:3000) and AbC22 (1:3000) directed against the C-terminal epitopes of $GABA_{B(1a/b)}$ and $GABA_{B(2)}$ subunits respectively. Secondary antibody was peroxidase coupled (1:3000). Blots were washed following each antibody incubation for 30 min with NETG. Detection of the antibody was performed using an enhanced chemiluminescence western blot analysis system (Amersham). Quantification of immunoblots was performed with Imagequant soft.

In all immunoblotting experiments a monoclonal antibody directed at α-syntaxin (1:3000) was used to ensure comparable protein load (Malitschek et al., 1998). The neuronal marker α-syntaxin was selected because it has been shown to be abundantly expressed in pituitary tissue (Majo et al., 1998) and there is no documentation on changes of syntaxin expression during development in the pituitary. Image quantification for the $GABA_B$ and the syntaxin bands was always done from the same Hyperfilm ECL (Amersham Lifesciences). The syntaxin bands had to be overexposed to reveal the low level of $GABA_B$ receptor protein in pituitary tissue. Consequently, the image "quantification" can only give a qualitative measure of the relative abundance of $GABA_B$ receptor subunit proteins and will not reliably detect subtle differences in protein expression levels.

2.4. Radioligand binding assay

The radioreceptor assay was performed essentially as described by Bowery et al. (1985) with minor modifications. For Scatchard studies, membranes were thawed and kept at room temperature for 30 min and then resuspended in a final concentration of approximately 6 mg of original tissue in 200 μl buffer (50 mM Tris–HCl; 2.5

mM $CaCl_2$, pH 7.4). Membrane preparations of 4-day-old, 20-day-old and adult male and female rats were incubated with ^{3}H-baclofen (NEN, specific activity 38.7 Ci/mm, final concentration range of 12.5–200 nM) and 50 μl buffer (for maximum binding) or 1 mM unlabeled L-baclofen (for non-specific binding). The incubation was performed for 40 min at 20°C and was terminated by rapid filtration on Whatman GF/B glass fiber filters, which were washed twice with ice-cold buffer. Radioactivity was counted in a beta counter. For saturation analysis studies, the incubation was performed with 200 nM of ^{3}H-baclofen, using the same membrane preparations and unlabeled baclofen concentration.

2.5. Statistical analysis

Sexual and ontogenic differences in $GABA_B$ receptor subunit expression were analyzed by two-way analysis of variance followed by Duncan's test (Statistica).

Scatchard analysis of binding data was performed by a computer curve-fitting program (Ligand) for a single class of binding sites. Changes in receptor number (B_{max}) and dissociation constants (K_d) among groups were analyzed using two-way analysis of variance followed by Duncan's test.

3. Results

3.1. Developmental expression of $GABA_B$ receptor subunits in anterior pituitary glands from female and male rats

Both $GABA_B$ subunits, $GABA_{B(1a)}$ and $GABA_{B(1b)}$, are expressed in developing female anterior pituitary glands from day 4 to adulthood (Fig. 1). The $GABA_{B(2)}$ subunit is barely detectable with the exception of proestrous membranes, where low levels could be observed (Fig. 1), but when quantified these were not significantly different from those of other ages (12-day-old, 0.07±0.02 vs. proestrus, 0.10±0.04, ns). Both $GABA_{B(1a)}$ and $GABA_{B(1b)}$ subunits are maximally expressed at 4 days of age and significantly decrease towards adulthood (Fig. 2). At days 4 and 12, $GABA_{B(1a)}$ is significantly more abundant than R1b; from day 20 onwards no significant difference in the expression of R1 subunits is observed in female pituitary membranes (Fig. 2).

In lactating females both subunits, $GABA_{B(1a)}$ and $GABA_{B(1b)}$, could be quantified, their expression being similar to proestrous females; while $GABA_{B(2)}$ could be detected in proestrous females, this subunit was not detectable in lactating rats.

The $GABA_{B(1a)}$ subunit is expressed in developing male anterior pituitary glands from day 4 to adulthood; by contrast, the $GABA_{B(1b)}$ subunit is barely detectable and not quantifiable at any of the ages studied (Fig. 3). As in females, the $GABA_{B(2)}$ subunit is barely detectable at any of the ages studied (Fig. 3). A significant decrease in the expression of the $GABA_{B(1a)}$ subunit throughout development was observed (Fig. 4), with the expression at day 4 significantly more abundant than at day 20 and onwards.

The expression of $GABA_B$ subunits in anterior pituitaries showed ontogenic sexual dimorphism: $GABA_{B(1a)}$ was significantly more abundant in 4- and 12-day-old females than in males (Fig. 4). On the other hand, while $GABA_{B(1b)}$ expression, though clearly lower than R1a, was detected throughout development in females, in males this subunit was barely detectable at any of the ages studied.

3.2. Radioligand binding assay of anterior pituitary $GABA_B$ receptors in developing male and female rats

^{3}H-Baclofen binds in a saturable manner to anterior pituitary membranes (Fig. 5, insets) at all ages studied and in both sexes. Scatchard plot analysis showed a single class of high-affinity binding sites (Fig. 5). K_d values in the different groups varied between 30 and 57 nM, similar to data from the literature for brain $GABA_B$ receptors (Bowery et al., 1985). No significant differences in K_d values were observed among different sexes or ages (Table 1). As observed by western blotting, the number of anterior pituitary binding sites of 4-day-old females was significantly higher than at 20 days of age or adulthood and also significantly higher than in 4-day-old males (Table 1).

In addition, 20-day-old males showed an increase in the number of binding sites with regard to 4-day-olds which was in the limit of significance ($p<0.06$).

In lactating rats the number of binding sites (31.4±5.4 fmol/mg protein, n=7) and the K_d (80.8±24 nM, n=3) were similar to those of proestrous female rats.

4. Discussion

This is the first demonstration of the ontogenic expression of $GABA_B$ receptor subunits in anterior pituitaries from developing and adult male and female rats. Some reports had shown $GABA_{B(1)}$ and $GABA_{B(2)}$ receptor subunit expression in rat melanotropes from the intermediate lobe (Morris et al., 1999; Shibuya et al., 1999). $GABA_{B(1a)}$ is the main subunit expressed in both male and female anterior pituitaries. $GABA_{B(1a)}$ expression gradually decreases throughout postnatal development, attaining the lowest levels in adult animals of both sexes. Furthermore, in females $GABA_{B(1a)}$ is significantly higher than in males at early stages of development. In addition, in females, $GABA_{B(1b)}$ expression also decreases throughout development and was much less abundant than $GABA_{B(1a)}$ at all ages studied. This is in

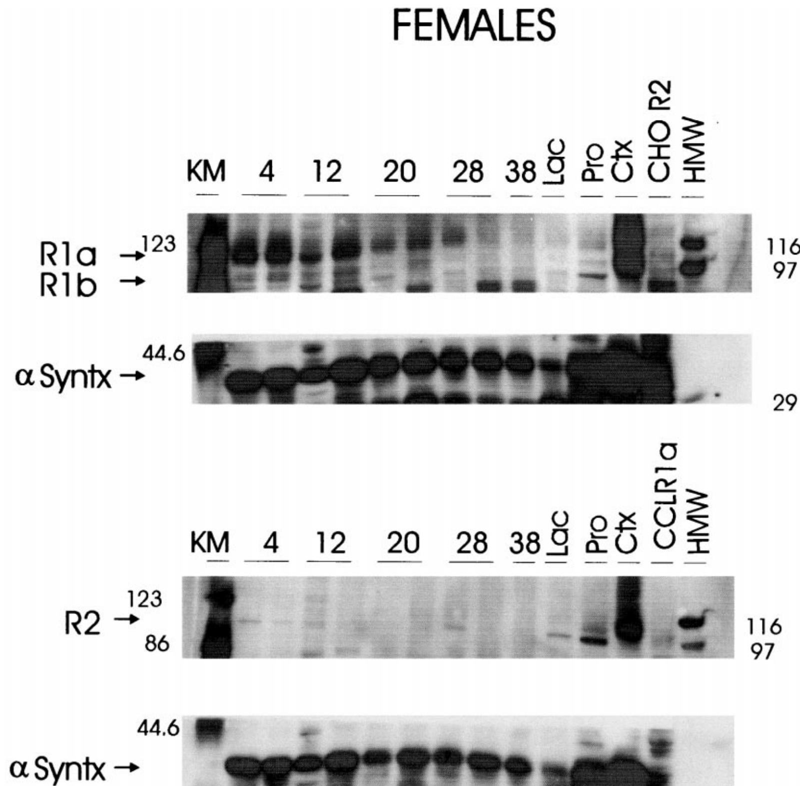

Fig. 1. Representative western blot of $GABA_B$ receptor subunits in female rat anterior pituitaries during development (ages 4, 12, 20, 28 and 38) and in adult lactating (Lac) and proestrous (Pro) rats. For this and the following figures: KM and HMW, molecular weight markers; Ctx, cortex positive control; CHO R2, CHO cells transfected with $GABA_{B(2)}$ subunit, negative control for R1 antibodies; CCLR1a, cells transfected with $GABA_{B(1a)}$ subunits, negative control for $GABA_{B(2)}$ antibodies; $GABA_{R1a}$ and R1b, bands immunostained with Ab 174.1 (130 KDa and 100 KDa respectively). $GABA_{R2}$, band immunostained with AbC22 (110 KDa); α Syntx (α-syntaxin, lower part of each blot, apparent molecular mass <45 kDa) is an internal control of protein load.

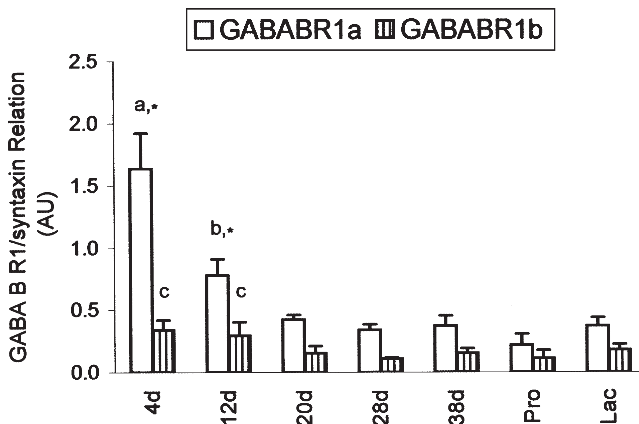

Fig. 2. Integration of $GABA_{B(1a)}$ and $GABA_{B(1b)}$ receptor subunit immunoblots of female developing and adult rat anterior pituitary membranes with Imagequant soft. Results in arbitrary units (AU) are the mean±SE of 4–5 independent samples and are expressed as the relation of each subunit with regard to the syntaxin control. a, significantly different from 12 days and onwards; b, significantly different from 20 days and onwards; c, significantly different from 20 days and onwards. Comparison between $GABA_{B(1a)}$ and $GABA_{B(1b)}$ expression in anterior pituitaries of developing and adult female rats: *, significantly different from $GABA_{B(1b)}$ at a certain age. For all cases, $p<0.05$ or less.

contrast to male rats where this subunit was barely detectable and not quantifiable. These results are in agreement with highest levels of expression of $GABA_{B(1)}$ subunits at early postnatal ages in some areas of the brain such as the spinal cord, cortex and cerebellum (Malitschek et al., 1998). $GABA_{B(1a)}$ was also the main subunit expressed at early postnatal ages in the cortex but, while in female pituitaries the substantial difference between $GABA_{B(1a)}$ and $GABA_{B(1b)}$ is lost in adulthood, $GABA_{B(1b)}$ remains the main subunit expressed in adult rat cortex (Malitschek et al., 1998). Similar results were observed in whole rat brain (Fritschy et al., 1999a,b).

The significant difference in intensity of $GABA_{B(1a)}$ expression between females and males and the lack of $GABA_{B(1b)}$ expression in males suggest a sexually dimorphic expression of $GABA_B$ subunits during ontogeny in the rat pituitary. Sexual differences in pituitary function and in central nervous system structures involved in the control of the adenohypophyseal secretion have been described extensively (Becu-Villalobos et al., 1997; Becu-Villalobos and Libertun, 1995) and point to the critical role of neonatal sexual steroids in these events.

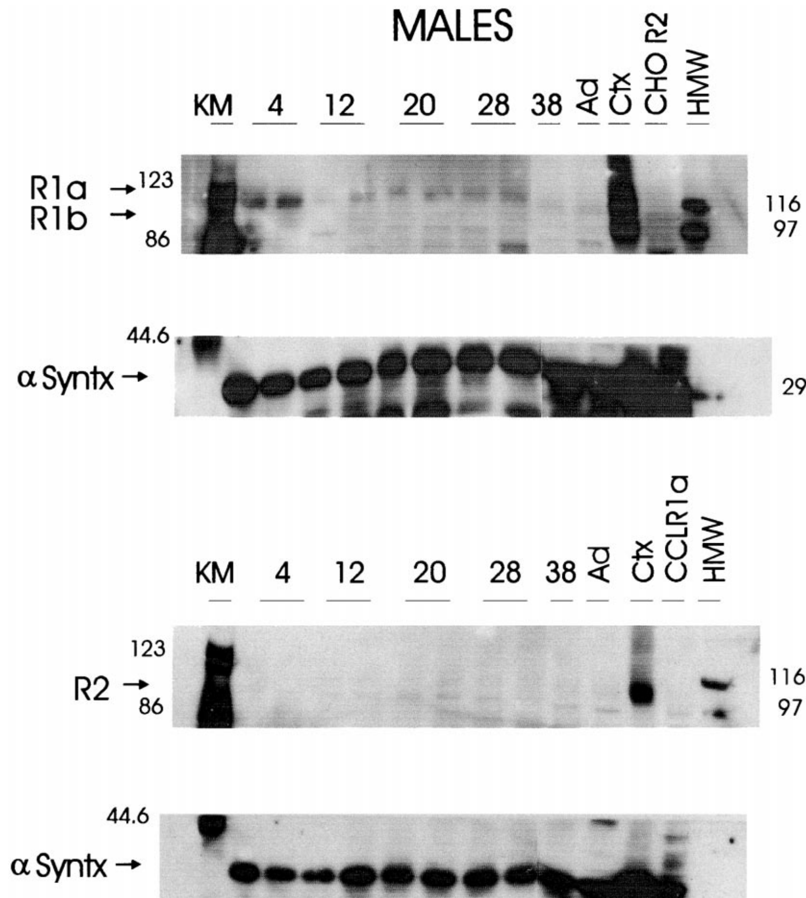

Fig. 3. Representative western blot analysis of $GABA_B$ receptor subunits in male rat anterior pituitaries throughout development (ages 4, 12, 20, 28 and 38) and in adult animals (Ad).

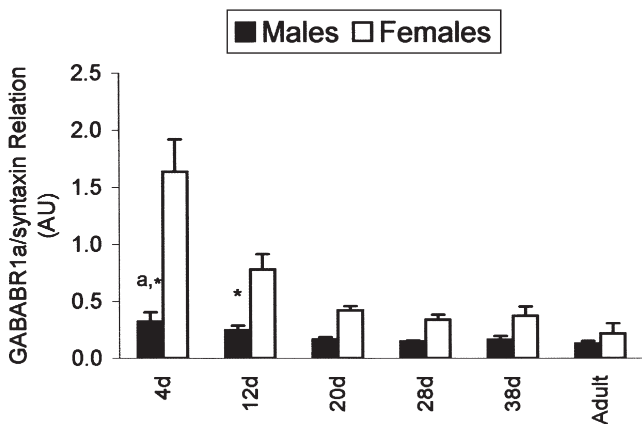

Fig. 4. Integration of $GABA_{B(1a)}$ receptor subunit immunoblots of male and female developing and adult rat anterior pituitary membranes with Imagequant soft. Results are the mean±SE of 4–5 independent samples: a, significantly different from 20 days and onwards; *, significantly different from females at a certain age.

$GABA_{B(2)}$ was barely detectable in adenohypophyseal membranes of either sex at any of the ages studied. The presence of $GABA_{B(1)}$ subunit expression in the absence of $GABA_{B(2)}$ has also been described in other tissues such as the human striatum (Martin et al., 1999) and the rat caudate putamen (Bowery, 1999; Clark et al., 2000). Very low levels of $GABA_{B(2)}$ expression were detected in the rat medial basal hypothalamus, septum and brainstem (Clark et al., 2000). The lack of $GABA_{B(2)}$ subunit detection raises the possibility that R_2 is expressed at very low levels, which are not detected by R_2 antibodies or that a yet to be identified $GABA_B$ receptor subunit or another associated protein may functionally complement $GABA_{B(1)}$ in the anterior pituitary, as $GABA_{B(1)}$ fails to reach the plasma membranes in the absence of $GABA_{B(2)}$ (Couve et al., 1998; Martin et al., 1999; Mohler and Fritschy, 1999; Ng et al., 1999). In this respect, it has been shown that $GABA_{B(1)}$ can interact specifically with additional proteins (Vernon et al., 1999).

Radioreceptor assays show that the adenohypophyseal $GABA_B$ receptor number significantly decreases during ontogeny in females and is more abundant in females than in males at early stages of development. This agrees well with our western blot results. While a temporal decrease in $GABA_B$ receptor number was observed in males by western blot analysis, this decrease was not detected by ^{3}H-baclofen binding studies. On the contrary, we observed a small increase in binding sites in males at P20 (postnatal day 20). A difference in the sen-

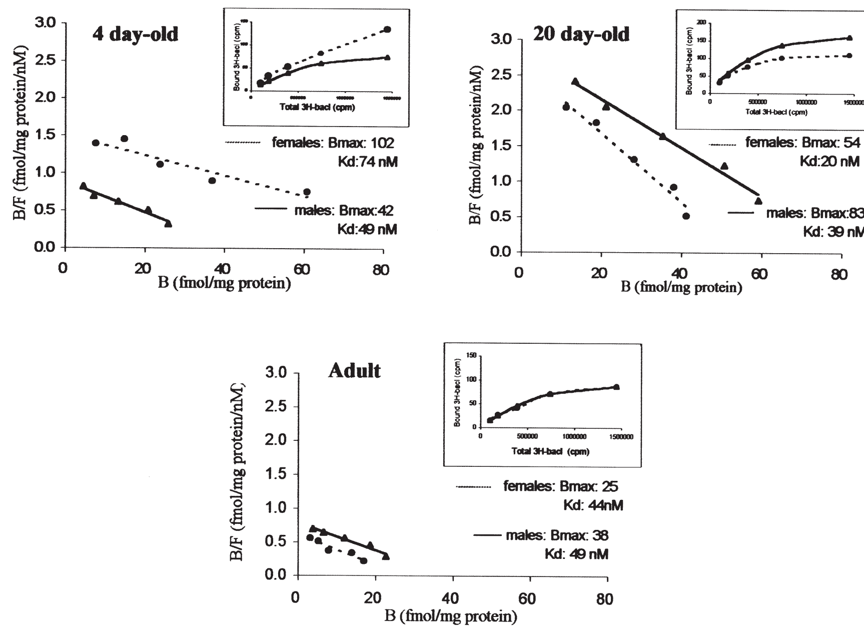

Fig. 5. Representative Scatchard plots of $GABA_B$ receptor binding studies in anterior pituitaries membranes of developing male and female rats. Insets show ligand specifically bound as a function of concentration (cpm).

Table 1
^{3}H-Baclofen binding to pituitary membranes of developing and adult male and female rats[a]

	B_{max} (fmol/mg protein)		
	4-day-old	20-day-old	Adult
Females	76.3±7.4 (6)* (K_d=57.1±8.0 nM)	42.8±6.7 (7) (K_d=37.2±7.1 nM)	29.1±4.6 (6) (K_d=48.1±3.3)
Males	33.5±5.6 (6) (K_d=38.8±4.5 nM)	54.4±6.4 (7)** (K_d=30.2±3.4 nM)	36.8±5.7 (6) (K_d=52.8±8.2 nM)

[a] B_{max} is the number of binding sites determined by saturation analysis. *Significantly different from 20-day-old and adult female rats and from 4-day-old and adult male rats, $p<0.05$. **Barely significant with respect to 4-day-old male rats, $p<0.06$. Number of samples given in parentheses. K_d values are the mean of three Scatchard plot experiments.

sitivity of the methods employed or, alternatively, the quantification detection in binding studies of other $GABA_B$ subunits (Isomoto et al., 1998) that are not detected with by the antiserum could account for this discrepancy.

On the other hand, the number of $GABA_B$ receptors in male adult rat anterior pituitary membranes determined in our studies is similar to that described by Anderson and Mitchell (1986). Anterior pituitary $GABA_B$ receptor K_d values described here are similar to central nervous system $GABA_B$ receptors (Bowery et al., 1985) and did not vary significantly with either sex or age. The lack of significant variation in K_d throughout development is in contrast with results in the cortex where the affinity was shown to gradually increase from birth to adulthood (Malitschek et al., 1998).

In summary, this study reports for the first time the expression of anterior pituitary $GABA_B$ receptor subunits. $GABA_{B(1a)}$ was the main subunit expressed; it decreased throughout postnatal development in both sexes and was more abundant in females than in males at early stages of development. $GABA_{B(1b)}$ was only detectable in females and followed a similar pattern. Surprisingly, detection of $GABA_{B(2)}$ expression was negligible in both sexes. No variations in $GABA_B$ receptor K_d values were observed along ontogeny. The precise cell type in which receptors are expressed, the protein which $GABA_{B(1)}$ possibly may interact with, in the absence of $GABA_{B(2)}$, and the implication of $GABA_B$ receptors in the regulation of hormone release at different stages of development in either sex will be the matter of further studies. A clear sexually dimorphic expression of $GABA_B$ receptor subunits was determined during ontogeny in the anterior pituitary. This event should be

studied in other central nervous system areas, in view of the vast divergence of neurological pathologies suffered by males and females.

Acknowledgements

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References

Anderson, R., Mitchell, R., 1986. Distribution of GABA binding sites subtypes in rat pituitary gland. Brain Research 365, 78–84.

Becu-Villalobos, D., Gonzalez Iglesias, A., Díaz-Torga, G., Hockl, P., Libertun, C., 1997. Brain sexual differentiation and gonadotropins secretion in the rat. Cellular and Molecular Neurobiology 17, 699–715.

Becu-Villalobos, D., Libertun, C., 1995. Development of gonadotropin-releasing hormone (GnRH) neuron regulation in the female rat. Cellular and Molecular Neurobiology 15, 165–176.

Bowery, N.G., 1996. Pharmacology of mammalian GABAB receptors. In: Enna, S., Bowery, N.G. (Eds.), The GABA receptors. Humana Press Inc, Totowa, NJ, pp. 1–30.

Bowery, N.G., 1999. Metabotropic GABAB receptors. Neurotransmissions 15, 3–11.

Bowery, N.G., Hill, D.R., Hudson, A.L., 1985. [3H](-)Baclofen: an improved ligand for GABAB sites. Neuropharmacology 24, 207–210.

Clark, J.A., Mezey, E., Lam, A.S., Bonner, T.I., 2000. Distribution of the GABA(B) receptor subunit gb2 in rat CNS. Brain Research 860, 41–52.

Couve, A., Filippov, A.K., Connolly, C.N., Bettler, B., Brown, D.A., Moss, S.J., 1998. Intracellular retention of recombinant GABAB receptors. Journal of Biological Chemistry 273, 26361–26367.

Duvilanski, B.H., Muñoz-Mainez, V., Díaz, M.D.C., Seilicovich, A., Debeljuk, L., 1984. Serum prolactin levels and GABA-related enzymes in the hypothalamus and anterior pituitary during maturation in the rat. Neuroendocrinology Letters 4, 243–251.

Ekstrand, J., Nyström, S., Emanuelsson, A., Nilsson, A.K., Johansson, T., Edlund, A. et al., 1999. Novel human GABAB receptor 1 isoforms. In: GABAB receptors. The Eighth Neuropharmacology Conference, Miami, USA. P3, 87.

Fiszer de Plazas, S., Becu, D., Mitridate de Novara, A., Libertun, C., 1982. GABA receptors in anterior pituitary and brain areas after median eminence lesions. Endocrinology 111, 1974–1978.

Fritschy, J.M., Giorgetta, O., Benke, D., 1999b. Differential maturation and cellular distribution of the GABAB receptor splice variants in developing brain. In: GABAB receptors. The Eighth Neuropharmacology Conference, Miami, USA. S-31, 51.

Fritschy, J.M., Meskenaite, V., Weinmann, O., Honer, M., Benke, D., Mohler, H., 1999a. GABAB-receptor splice varaints GB1a and GB1b in rat brain: developmental regulation, cellular distribution and extrasynaptic localization. European Journal of Neuroscience 11, 761–768.

Fritschy, J.M., Paysan, J., Enna, A., Mohler, H., 1994. Switch in the expression of rat GABAA-receptor subtypes during postnatal development: an immunohistochemical study. Journal of Neuroscience 14, 5302–5324.

Grandison, L., 1981. Anterior pituitary GABA receptors and their regulation of prolactin secretion. In: Costa, E., Di Chiara, G., Gessa, G.L. (Eds.), GABA and benzodiazepine receptors. Raven Press, New York, pp. 219–227.

Isomoto, S., Kibara, M., Sakurai-Yamashita, Y., Nagyama, Y., Uezono, Y., Yano, K. et al., 1998. Cloning and tissue distribution of novel splice variants of the rat GABAB receptor. Biochemical and Biophysical Research Communications 253, 10–15.

Jones, K.A., Borowsky, B., Tamm, J.A., Craig, D.A., Durkin, M.M., Dai, M. et al., 1998. $GABA_B$ receptors function as a heteromeric assembly of the subunits $GABA_BR1$ and $GABA_BR2$. Nature 396, 674–769.

Kaupmann, K., Huggel, K., Heid, J., Flor, P., Bischoff, S., Mickel, S. et al., 1997. Expression cloning of GABA B receptors uncovers similarity to metabotropic glutamate receptors. Nature 386, 239–246.

Kaupmann, K., Malitschek, B., Schuler, B., Heid, J., Froestl, W., Beck, P. et al., 1998. GABA B receptor subtypes assemble into functional heteromeric complexes. Nature 396, 683–687.

Kuner, R., Köhr, G., Grünewald, S., Eisenhardt, G., Bach, A., Kornau, H., 1999. Role of heteromer formation in GABAB receptor function. Science 283, 74–77.

Lacau-Mengido, I.M., Diaz-Torga, G.S., Libertun, C., 1989. Diazepam: endocrine effects and hypothalamic binding sites in the developing male and female rat. Life Sciences 45, 567–575.

Lux-Lantos, V., Rey-Roldán, E.B., Becu-Villalobos, D., Chamson-Reig, A., Bianchi, M.S., Libertun, C., 1999. Mechanism of action of GABA B receptors in anterior pituitary cells. In: GABAB receptors. The Eighth Neuropharmacology Conference, Miami, USA. P15, 99.

Lux-Lantos, V.A.R., Rey, E.B., Libertun, C., 1992. Activation of GABA B receptors in the anterior pituitary inhibits prolactin and luteinizing hormone secretion. Neuroendocrinology 56, 687–693.

Majo, G., Aguado, F., Blasi, J., Marsal, J., 1998. Synaptobrevin isoforms in secretory granules and synaptic-like microvesicles in anterior pituitary cells. Life Sciences 62, 607–616.

Malitschek, B., Rüegg, D., Heid, J., Kaupmann, K., Bittiger, H., Froestl, W. et al., 1998. Developmental changes of agonist affinity at $GABA_BR1$ receptor variants in the rat. Molecular and Cellular Neuroscience 12, 56–64.

Martin, S.C., Russek, S.J., Farb, D.H., 1999. Molecular identification of the human GABABR2: cell surface expression and coupling to adenylyl cyclase in the absence of GABABR1. Molecular and Cellular Neuroscience 13, 180–191.

Moguilevsky, J.A., Carbone, S., Szwarcfarb, B., 1992. Changes in the effect of gamma-aminobutyric acid on prolactin secretion during sexual maturation in female rats. Endocrinology 131, 458–462.

Mohler, H., Fritschy, J.M., 1999. GABAB receptors make it to the top—as dimers. Trends in Pharmacological Science 20, 87–89.

Morris, S.J., Davis, T.D., Wolfe, S.E., Beatty, D.M., Enna, S.J., Low, M.J. et al., 1999. A murine melanotrope-derived cell line expressing endogenous GABAB receptors. In: GABAB receptors. The Eighth Neuropharmacology Conference, Miami, USA. P11, 95.

Ng, G.Y., Clark, J., Coulombe, N., Ethier, N., Hebert, T.E., Sullivan, R. et al., 1999. Identification of a GABAB receptor subunit, gb2, required for functional GAGAB receptor activity. Journal of Biological Chemistry 274, 7607–7610.

Olpe, H.R., Karlsson, G., Pozza, M.F., Brugger, F., Steinmann, M., van Riezen, H. et al., 1990. CGP 53348: a centrally active blocker of GABAB receptors. European Journal of Pharmacology 187, 27–38.

Pfaff, T., Malitschek, B., Kaupmann, K., Prézeau, L., Pin, J.-P., Bettler, B. et al., 1999. Alternative splicing generates a novel isoform of the rat metabotropic $GABA_BR1$ receptor. European Journal of Neuroscience 11, 2874–2882.

Rey-Roldán, E.B., Lux-Lantos, V., Chamson-Reig, A., Libertun, C., 1997. In vivo interaction of baclofen, TRH and serotonin on PRL

and TSH secretion in the developing and adult male and female rats. Life Sciences 61, 2283–2290.

Rey-Roldán, E.B., Lux-Lantos, V.A.R., Gonzalez Iglesias, A., Becu-Villalobos, D., Libertun, C., 1996. Baclofen, a gamma-aminobutyric acid B agonist, modifies hormonal secretion in pituitary cells from infantile female rats. Life Sciences 58, 1059–1065.

Shibuya, I., Harayama, N., Sasaki, N., Uezono, Y., Tanaka, K., Ueta, Y. et al., 1999. Distinct cellular mechanisms utilized by GABAB and dopamine D2 receptors in suppressing Ca2+ signalling of pituitary melanotrophs. In: GABAB receptors. The Eighth Neuropharmacology Conference, Miami, USA. P23, 109.

Turgeon, S.M., Albin, R.L., 1994. Postnatal ontogeny of GABAB binding in the rat brain. Neuroscience 62, 601–613.

Vernon, E., Meyer, G., Pickard, L., Noel, J., Collingridge, G.L., Henley, J.M., 1999. Identification and characterization of a protein which interacts with the GABABR1 subunit. In: GABAB receptors. The Eighth Neuropharmacology Conference, Miami, USA. S21, 45.

White, J., Wise, A., Main, M., Green, A., Fraser, N., Disney, G. et al., 1998. Heterodimerization is required for the formation of a functional GABAB receptor. Nature 396, 679–682.