Original Paper

Targeted Ablation of Gonadotrophs in Transgenic Mice Depresses Prolactin but Not Growth Hormone Gene Expression at Birth as Measured by Quantitative mRNA Detection

Hugo Vankelecom   Eve Seuntjens   Annelies Hauspie   Carl Denef

Department of Molecular Cell Biology, Laboratory of Cell Pharmacology, University of Leuven (K.U. Leuven),
Medical School, Campus Gasthuisberg, Leuven, Belgium

Abstract

We previously reported that transgenic ablation of go-
nadotrophs results in impaired development of cells
immunostainable for prolactin (PRL) but not of cells
immunostainable for growth hormone (GH) or pro-
opiomelanocortin (POMC) in pituitary of newborn mice.
The question remained whether this reduction in PRL
protein is a reflection of reduced PRL mRNA expression,
or whether this regulation is only situated at the transla-
tional level. We therefore generated a new series of
transgenic mice in which gonadotrophs were ablated by
diphtheria toxin A targeting, and analyzed hormone
mRNA levels instead of hormone protein around the day
of birth. Pituitary mRNA expression levels of luteinizing
hormone-β (LHβ), PRL and GH were quantified using
real-time TaqMan RT-PCR. Of the 13 transgenic mice
obtained, 8 showed a clear-cut reduction (ranging from
62 to 98%) in LHβ mRNA levels. PRL mRNA values were
significantly reduced in the transgenic mice (p = 0.0034),
while GH mRNA expression was unaffected (p = 0.93).
An additional observation was that female newborn
mice produce 5 times more LHβ mRNA than male mice
whereas no sex difference was observed for expression
levels of PRL and GH mRNA. Moreover, in the wild-type
mice, LHβ mRNA expression was 20-fold higher than GH
mRNA expression which in turn was 500- to 1,000-fold
higher than PRL mRNA expression, suggesting a low
expression level of the PRL gene at birth. In conclusion,
the present data support the hypothesis that embryonic
development of PRL gene expression is stimulated by
gonadotrophs.

Introduction

Various in vitro observations suggest a paracrine stim-
ulatory action of gonadotrophs on lactotroph secretion,
cell growth and PRL gene expression [2, 3, 15, 33, 34]. In
recent work, we obtained experimental data suggesting a
developmental interaction between gonadotrophs and
lactotrophs also in vivo [31]. Transgenic ablation of go-
nadotrophs in mice by targeted expression of diphtheria
toxin A (DTA) in gonadotrophs using the glycoprotein
hormone α-subunit (αSU) promoter, reduces embryonic

development of cells expressing PRL (lactotrophs), but not of cells expressing GH (somatotrophs) or pro-opiomelanocortin (POMC) (corticotrophs), assessed at the level of hormone protein immunoreactivity. The study was performed on newborn mice because estrogen secretion in blood is negligible at that developmental stage, ruling out interference of estrogens in the observed effect of gonadotroph ablation [31].

The question remained whether control of PRL gene expression through gonadotrophs in vivo was at the translational or mRNA expression level. Recent new findings emphasized the relevance of the latter question. By means of reverse-transcription polymerase chain reaction (RT-PCR) analysis for hormone mRNA content in single pituitary cells from P1 (postnatal day 1) mice, it was found that classic PRL cells (monohormonal PRL cells) are very rare in newborn mice and that most cells expressing PRL mRNA in newborn mice (more than 70% of all PRL mRNA-expressing cells) also express mRNA of other hormones, including growth hormone (GH) and POMC, in different combinations [30]. In view of these findings, it was not understood why the decrease in number of lactotrophs by gonadotroph ablation reported previously, was not accompanied by a decrease in the number of somatotrophs and corticotrophs, except when it was assumed that there was a selective effect on PRL gene expression. Thus, it seemed important to reexamine the role of gonadotrophs on lactotroph development by means of detection of hormone mRNA instead of immunostaining of the hormone protein. Therefore, new gonadotroph-ablated transgenic mice were produced and PRL, GH and luteinizing hormone-β (LHβ) mRNA levels in pituitary RNA extracts were quantified by real-time TaqMan RT-PCR. In addition, this analysis would give information as to whether control by gonadotrophs is exerted at the mRNA expression and/or translational level.

Materials and Methods

Reagents

AmpliTaq Gold and PCR reagents were obtained from Applied Biosystems (Foster City, Calif., USA), first-strand buffer, DTT, Moloney murine leukemia virus (M-MLV) reverse transcriptase, and RNase-free DNase I from Life Technologies (Grand Island, N.Y., USA), recombinant RNasin ribonuclease inhibitor from Promega (Madison, Wisc., USA), and TaqMan probes from Eurogentec (Seraing, Belgium). Oligonucleotide primers were bought from AP Biotech (Uppsala, Sweden), and Tripure reagent from Roche Diagnostics (Mannheim, Germany). RiboGreen RNA quantitation kit came from Molecular Probes (Eugene, Oreg., USA).

Transgenic Mice

Transgenic mice were produced using the αSU-DTA construct by zygote microinjection as described in detail before [31]. The construct contains DTA under control of the –313/+48 bovine αSU promoter shown to target expression of a transgene to gonadotrophs but not to thyrotrophs [4, 13, 17, 24, 31]. F_0 ('founder') αSU-DTA mice were studied since gonadotroph ablation precludes the establishment of a transgenic line due to infertility. All animal experiments were conducted in accord with the 'Guidelines for Care and Use of Experimental Animals' as signed by the Endocrine Society, and approved by the University Ethical Committee.

Quantitative Real-Time RT-PCR (TaqMan) on Pituitary RNA from Transgenic αSU-DTA Mice and Wild-Type Control Mice

Pups from microinjected zygotes were killed by decapitation around the day of birth (= P1, postnatal day 1), i.e. between P0 (= delivery by cesarian section on the calculated day of birth) and P3. Different perinatal ages were analyzed to explore possible rapid changes in hormone mRNA expression levels. Delivery by cesarian section was sometimes done to prevent the pups from being killed by the mother immediately after birth, thus being lost for analysis. The pituitary gland was isolated under the dissecting microscope, and transferred to 500 μl Tripure reagent for RNA isolation. The final RNA extract (4 μl) was treated with DNase (0.5 U) for 20 min at 25°C followed by enzyme inactivation at 65°C for 10 min in the presence of 2.5 mM EDTA. 0.5 μl of RNA extract (out of 5.5 μl) was used for RNA quantification using the Ribogreen Quantitation reagent. Completeness of DNase treatment was checked by RT-PCR of GH with and without M-MLV reverse transcriptase in each sample. In all tests, GH amplicons were not observed when M-MLV reverse transcriptase was omitted from the RT step. An additional safeguard to exclude genomic DNA amplification during the real-time PCR step was provided by choosing primers or probes spanning an intron (table 1).

100 ng of total pituitary RNA, or the whole RNA sample if less than 100 ng was available, was reverse-transcribed in a final volume of 20 μl using the following conditions: 1× first-strand buffer, 1 nM DTT, 20 U of RNasin, 500 μM of each dNTP, 5 μM random hexamers, and 200 U of M-MLV reverse transcriptase. Part of this mixture was heated for 10 min at 70°C and immediately cooled on ice. Then, dNTPs, random hexamers and M-MLV reverse transcriptase were added, and the final mixture subjected to the following cycle: 15 min at 25°C, 50 min at 42°C, and 10 min at 95°C before cooling down to 4°C. Samples were kept at –20°C, or immediately processed by TaqMan PCR. Samples were assayed in tri- or quadruplicate, and reactions without target cDNA were used as negative controls. 18S rRNA (TaqMan Ribosomal RNA Control Reagents – VIC Probe; Applied Biosystems) was used as an internal standard for normalization of hormone mRNA amount in each reaction. Primers and TaqMan probes were designed from GenBank sequences using Primer Express 1.0 Software (Applied Biosystems), and are summarized in table 1. The principles of the technique are described in Orlando et al. [22]. Reaction mixtures (final volume: 25 μl) containing between 0.002 and 15 ng reverse-transcribed RNA, oligonucleotide primers at a final concentration of 900 nM for hormones and 50 nM for 18S rRNA (following the manufacturer's recommendations), TaqMan probe at a final concentration of 200 nM and 1× TaqMan Universal PCR Master Mix (Applied Biosystems) were incubated in a 96-well plate on an ABI PRISM 7700 Sequence Detector (Applied Biosystems) using the following temperature cycling: 10 min at 95°C for

Table 1. Nucleotide sequence of TaqMan PCR primers and probes used in real-time PCR to quantify GH, PRL and LHβ cDNA levels

| cDNA | TaqMan PCR primers and probes | Position in GenBank sequence | |
	Sequence[a]	accession No.	position
GH	S: 5'-TAATGCTGTGCTCCGAGCC-3'	X02891	172–190
	AS: 5'-GAATGGAATAGCGCTGTCCC-3'		253–272
	P: 5'-TGACACCTACAAAGAGTTCGAGCGTGCCT-3'		214–242
PRL	S: 5'-GGGTCAGCCCAGAAAGCAG-3'	X04418	67–85
	AS: 5'-CAGTCACCAGCGGAACAGATT-3'		147–167
	P: 5'-CTGCTGTTCTGCCAAAATGTTCAGCCTCT-3'		115–143
LHβ	S: 5'-CGGCCTGTCAACGCAACT-3'	Y10418	39–56
	AS: 5'-GGCAGTACTCGGACCATGCT-3'		129–148
	P: 5'-TGAGTTCTGCCCAGTCTGCATCACCTTC-3'		71–98

[a] S = Sense primer; AS = antisense primer; P = probe, labeled at the 5' end with 6-FAM (6-carboxyfluorescein), and at the 3' end with TAMRA (6-carboxytetramethylrhodamine).

activation of AmpliTaq Gold DNA polymerase, followed by 40 cycles of 15 s at 95 °C for denaturation and 1 min at 60 °C for annealing and amplification. In each plate, serial dilutions of adult mouse pituitary cDNA were used as template under the same conditions (same sets of primers and probe) to generate standard curves relating the threshold cycle (C_t) to the log input amount of cDNA. The relative amounts of hormone mRNA (cDNA) in each sample were determined using the standard curve method, as described in detail in ABI PRISM Sequence Detection System User Bulletin 2 (Applied Biosystems) and in Fink et al. [10], and were then normalized to 18S rRNA (cDNA) levels.

Real-time RT-PCRs were done to measure LHβ, PRL and GH mRNA levels. The amount of RNA recovered from a neonatal pituitary was limited and did not allow quantification of additional hormone mRNAs.

Conversion factors, based on the amount of template included in the reaction and on the sensitivity of the assays for the individual hormones, were applied to the values obtained in order to compare (normalized) expression levels of the three hormones. Standard curves with serial dilutions of purified hormone cDNA revealed that the TaqMan PCR for GH was 50-times more sensitive than for PRL and LHβ (data not shown).

Statistical Analysis

Linear mixed models were used to compare the hormone expression levels in control and transgenic mice [35]. All mice were included in the analysis, also the transgenic mice in which no ablation had occurred (evident from absence of a significant decrease in LHβ mRNA values). The model takes into account the dependence between mice from the same litter, and contains group (control/transgenic), age and gender as fixed effects. Approximate F-tests were used to evaluate the effect of group, age and gender. For LHβ mRNA, a logarithmic transformation was needed to meet the assumptions of the analysis.

In a further analysis, an attempt was made to explore the relation between the 'change' in LHβ mRNA levels and the 'change' in PRL or GH mRNA levels. As 'change', we used the difference in hormone mRNA expression level between a transgenic mouse and its control mice (paired as grouped in table 2), since a 'real change' (i.e. difference between hormone mRNA expression level before and after ablation in a transgenic mouse) obviously could not be measured. If more than one control mouse was available, the mean of the measurements of these controls was used to calculate the difference with the measurement of the transgenic mouse. In this way, 13 'changes' in LHβ mRNA levels and 13 'changes' in PRL or GH mRNA levels were obtained. A regression model was used to analyze the relation between both 'changes', after correction for the effect of gender. Since the quality of the controls was not the same for each transgenic mouse (e.g. not the same age, not the same litter), conclusions based on this analysis should be considered as tentative. A logarithmic transformation of the 'change' in LHβ mRNA was needed to fit in the regression model.

To compare the levels of LHβ, PRL and GH mRNA in the control mice, the hormone mRNA expression value was treated as a repeated factor. This statistical technique was needed to take into account the dependence of the three hormone mRNA expression values which are measured in each (individual) mouse and, therefore, are not independent. A logarithmic transformation of the hormone mRNA expression levels was needed.

All analyses were performed with the statistical package SAS (version 8.1).

Results

Gonadotroph depletion was achieved in transgenic αSU-DTA mice. LHβ mRNA levels were quantified in pituitary RNA from perinatal (P0–P3) pups obtained by zygote microinjection to score the degree of gonadotroph ablation. Of the 13 transgenic mice obtained (out of 158

Table 2. Overview of pituitary LHβ, PRL and GH mRNA expression levels in perinatal transgenic αSU-DTA mice and their wild-type controls

Mouse No.	Sex	Wt/tg[b]	Age[c]	Hormone mRNA levels[d]		
				LHβ	PRL	GH
47,1	m	wt	P0	96.7	0.0190	11.01
43,1	m	wt	P0	81.5	0.0111	9.75
43,3[a]	m	tg	P0	9.6	0.0019	10.18
91,2	m	wt	P1	113.6	0.0255	15.08
91,4	m	wt	P1	96.9	0.0277	20.12
91,1	m	tg	P1	113.3	0.0261	21.12
107,3	m	wt	P1	143.6	0.0284	14.56
107,4	m	wt	P1	122.0	0.0260	21.05
107,5[a]	m	tg	P1	31.2	0.0101	17.12
83,1	m	wt	P1	128.6	0.0233	15.16
83,4	m	wt	P1	111.0	0.0206	21.99
112,1	m	tg	P1	126.0	0.0200	16.02
67,1	m	wt	P1–P2	239.6	0.0186	13.34
67,2	m	wt	P1–P2	241.6	0.0232	12.66
68,1[a]	m	tg	P1–P2	8.1	0.0042	14.66
11,2	m	wt	P2	217.6	0.0622	12.20
11,1	m	tg	P2	164.8	0.0275	15.01
26,1	m	wt	P2	27.0	0.0102	29.01
26,2[a]	m	tg	P2	3.4	0.0024	24.40
40,3	m	wt	P2	215.3	0.0717	10.01
40,4	m	wt	P2	248.6	0.0792	9.56
95,2[a]	m	tg	P2	5.5	0.0208	10.95
47,3	f	wt	P0	1291.1	0.0291	7.08
47,2[a]	f	tg	P0	492.0	0.0080	6.83
51,6	f	wt	P1	416.0	0.0063	19.51
51,7	f	wt	P1	421.6	0.0062	18.82
51,5	f	tg	P1	296.7	0.0052	19.05
115,1	f	wt	P1	1148.7	0.0293	20.49
115,2	f	wt	P1	1129.2	0.0248	26.23
115,4[a]	f	tg	P1	165.5	0.0045	24.06
115,5	f	tg	P1	887.6	0.0215	20.39
38,1	f	wt	P3	604.7	0.0292	11.07
38,2[a]	f	tg	P3	172.5	0.0059	13.85

[a] Mice showing a clear-cut reduction of pituitary LHβ mRNA expression.
[b] Wt = Wild type; tg = transgenic.
[c] P1 = day of birth (= postnatal day 1); P0 = delivery by cesarian section on the prospective day of birth; P1–P2 means that day of birth is uncertain for 1 day.
[d] Hormone mRNA levels in the pituitary were quantified by real-time TaqMan RT-PCR, and normalized to 18S rRNA levels. Ratios were multiplied by 1,000. In addition, conversion factors were applied to the values obtained in order to compare expression levels of the three hormones.

Fig. 1. Effect of transgenic gonadotroph ablation on pituitary LHβ (**a**), PRL (**b**) and GH (**c**) mRNA expression levels in perinatal mice. Gonadotroph ablation in αSU-DTA mice causes a significant reduction in both LHβ mRNA levels and PRL mRNA expression. GH mRNA expression levels are not affected. Control mice, n = 20; αSU-DTA mice, n = 13. Bars represent SE. * Statistically significant difference in αSU-DTA versus control mice (for p values, see Results).

mice born), 8 mice (marked with an asterisk in table 2) showed a 62–98% reduction in LHβ mRNA levels. Statistical analysis using linear mixed models [35] revealed significantly lower LHβ mRNA levels in transgenic mice (p = 0.002 after correction for gender; fig. 1). PRL mRNA levels were also significantly reduced in αSU-DTA mice (p = 0.0034; fig. 1), while GH mRNA expression was unaffected (p = 0.93; fig. 1). These differences were neither influenced by age of the animals (i.e. P0 to P3; for
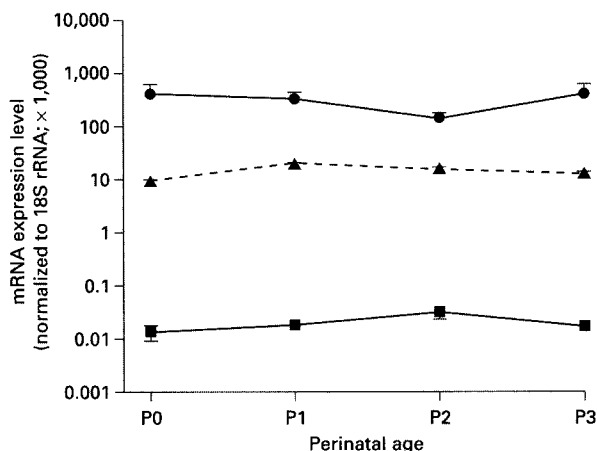
Fig. 3. Pituitary LHβ, PRL and GH mRNA expression levels in mice at different perinatal ages. Expression levels of LHβ (●), PRL (■) and GH (▲) mRNA do not significantly change between the day of birth (P0 = delivery by cesarian section on the prospective day of birth; P1 = day of birth or postnatal day 1) and postnatal day 2 or 3 (P2 and P3, respectively). Values of P1–P2 (table 2) were included in the mean of P2. mRNA expression levels are shown on a log scale. Control + αSU-DTA mice: P0, n = 5; P1, n = 16; P2, n = 10; P3, n = 2. Bars represent SE.

Fig. 2. Pituitary LHβ (**a**), PRL (**b**) and GH (**c**) mRNA expression levels in female and male perinatal mice. LHβ mRNA expression levels in female pituitaries are 5-fold higher than in male pituitaries. No significant differences are measured for PRL and GH mRNA expression levels. Male mice (control + αSU-DTA), n = 22; female mice (control + αSU-DTA), n = 11. Bars represent SE. * Statistically significant difference in female versus male mice (for p values, see Results).

PRL: p = 0.23; for GH: p = 0.96), nor by gender (for PRL: p = 0.48, and GH: p = 0.94). The extent of PRL mRNA reduction in the transgenic mice was correlated with the degree of LHβ mRNA reduction; 'changes' in LHβ mRNA levels (i.e. differences between control and transgenic mice) were positively related (p = 0.001 after correction for gender) to 'changes' in PRL mRNA values (partial correlation coefficient = 0.71), whereas no significant relation (p = 0.78 after correction for gender) was ob-

served between 'changes' in LHβ and GH mRNA levels (partial correlation coefficient = –0.11).

An additional observation was that LHβ mRNA levels were significantly higher (~ 5-fold) in female than in male perinatal mice (statistical analysis within the whole group of transgenic and control mice; p < 0.0001 after correction for group, i.e. transgenic or control; fig. 2). PRL and GH mRNA levels were not different between newborn male and female mice (p = 0.63 and 0.61, respectively; fig. 2). Differences in PRL mRNA expression levels measured at the different ages P0 to P3 were at the border of non-significance (p = 0.05; fig. 3). There was no significant difference in GH mRNA level between the different ages (p = 0.21; fig. 3), neither was there a significant difference in LHβ mRNA content (after correction for gender and group; p = 0.64; fig. 3).

Analyses within the group of control mice proper further revealed that at the perinatal age LHβ mRNA expression levels were significantly higher (~ 20-fold) than GH mRNA expression levels (p < 0.0001; fig. 4), and GH mRNA levels were 500- to 1,000-fold higher than PRL mRNA levels (p < 0.0001; fig. 4).

Gonadotrophs and Development of
Prolactin Gene Expression

Fig. 4. Pituitary LHβ, PRL and GH mRNA expression levels in normal (wild-type) perinatal mice. In the pituitaries of perinatal wild-type mice, LHβ mRNA expression levels are 20-fold higher than GH mRNA expression levels. PRL mRNA levels are 500- to 1,000-fold lower than GH mRNA levels. mRNA expression levels are shown on a log scale. For all hormones, n = 20 (wild-type mice = control mice). Bars represent SE. * p < 0.0001 for GH versus LHβ mRNA level; ** p < 0.0001 for PRL versus GH mRNA level.

Discussion

In the present study we show that ablation of gonadotrophs during embryonic development results in a reduced PRL, but not GH, mRNA expression level in the pituitary of perinatal mice. These results are an extension to our previous study [31] in which impaired development of PRL cells, scored by immunoreactivity to anti-PRL antiserum, was found in transgenic mice with gonadotroph ablation.

Previous immunocytochemical findings were challenged by more recent observations. We found that in normal pituitary, a substantial number of cells express different pituitary hormone genes at the mRNA level, including PRL, GH and POMC, not only in adult life but also during fetal and neonatal life [25, 30]. The proportion of cells with specific combinations of hormone mRNA was particularly high compared with monohormonal PRL mRNA cells in P1 mice. If most cells containing PRL mRNA also contain either GH and/or POMC mRNA during early life, it is unclear why in the gonadotroph-ablated mice only the PRL-immunoreactive cells were decreased and not the number of GH- and POMC/ACTH-immunoreactive cells. The present findings show

that in these gonadotroph-ablated neonatal mice PRL mRNA expression levels are significantly reduced compared to wild-type controls, while GH mRNA levels are unchanged. Therefore, gonadotrophs seem to control the number of developing lactotrophs by selectively stimulating PRL gene expression, not only in monohormonal PRL cells, but also in plurihormonal PRL cells where GH expression is not affected. What may be the basis of the selective interference with PRL mRNA expression? PRL and GH gene activation are both crucially dependent on the POU homeodomain transcription factor GHF-1/Pit-1 [28, 29]. Confinement of expression to the proper cell type requires combinatorial interactions of Pit-1 with other (transcription) factors [28, 29, 32]. Lactotroph-specific expression of PRL needs interaction of Pit-1 with Ets-1 (a widely expressed proto-oncogene product) on the PRL promoter through binding of a composite Ets-1/Pit-1 cis-acting element, not present in the GH gene [6]. Likewise, efficient GH gene expression is dependent on synergistic combinations of Pit-1 with factors such as Zn-15 (member of the Cys/His zinc finger family of transcription factors) [18] and the thyroid hormone receptor [27]. Another Pit-1-dependent hormone gene, TSHβ, is activated by cooperative interaction of GATA-2 and Pit-1, which both bind to the TSHβ promoter [11]. In addition to these combinatorial codes of transcription factors, recruitment of coactivator – or corepressor – complexes seems to be fundamental to the selective activation or repression of the PRL and the GH gene by Pit-1 in lactotrophs and somatotrophs [28, 29]. The selective effect of the gonadotroph-derived factor(s) on PRL mRNA expression as reported here suggests a unique interference with signaling pathways that lead to PRL gene activation but not to GH gene transcription. One example of such a PRL-specific signal transduction pathway is the Ras-dependent activation of the Ets-1/Pit-1 synergy in PRL (but not GH) gene activation, where essential coactivators are recruited to the PRL gene [5, 6, 16, 28, 29, 36]. Gonadotroph-derived paracrine factors may act through this pathway to selectively affect PRL expression. Candidate paracrine factors that affect lactotroph growth and PRL production are αSU, N-POMC-derived peptides and certain growth factors (EGF, TGFα) (reviewed in [7]). Some of these factors (e.g. EGF) have been demonstrated to act through a Ras-dependent pathway [23].

It remains unclear, however, whether transcription factor combinations that govern specific hormone gene activation in monohormonal cells are identical to the combinatorial codes, if any, used in plurihormonal cells.

In the present study, quantitative RT-PCR revealed some additional findings. It was noted that neonatal PRL mRNA levels were a 1,000 times lower than GH mRNA levels. Low PRL levels at birth are in line with other studies in mice and rats [1, 20]. On the other hand, PRL levels in pigs and sheep have been reported to be rather high around birth [19, 26]. Another interesting finding was that on average female newborn mice produce 5 times more LHβ mRNA than males. Sex-dependent differences of LH production in newborn mice have not been documented before. Moreover, pituitary and plasma LH levels in wild-type mice seem to be very low at birth in both sexes [8, 20], which is not in accordance with the high mRNA levels we measured both in female and male mice. Protein levels, however, do not necessarily correlate with mRNA expression levels [12, 14, 21], and our data therefore suggest an interesting difference in regulation of LHβ mRNA and protein expression in the neonatal pituitary. High LH gene activation may coincide with the onset of pulsatile GnRH secretion in rodents towards the end of gestation [9].

In conclusion, new data on PRL gene expression in gonadotroph-ablated newborn mice using mRNA levels as a hallmark confirm our previous observations on selectively impaired development of PRL cells when gonadotrophs are depleted. Gonadotrophs may increase the number of cells expressing PRL mRNA or may increase the PRL mRNA level in already existing cells that express PRL alone (classic lactotrophs) or coexpress PRL together with GH or POMC. Furthermore, the present data indicate that control of lactotroph development by gonadotrophs is at least in part at the PRL mRNA level, and not, or not only, at the PRL protein level.

References

1 Barkley MS. Serum prolactin in the male mouse from birth to maturity. J Endocrinol 83: 31–33;1979.

2 Bégeot M, Hemming FJ, Dubois PM, Combarnous Y, Dubois MP, Aubert ML. Induction of pituitary lactotrope differentiation by luteinizing hormone alpha subunit. Science 226:566–568;1984.

3 Bégeot M, Hemming FJ, Martinat N, Dubois MP, Dubois PM. Gonadotropin releasing hormone (GnRH) stimulates immunoreactive lactotrope differentiation. Endocrinology 112: 2224–2226;1983.

4 Bokar JA, Keri RA, Farmerie TA, Fenstermaker RA, Andersen B, Hamernik DL, Yun J, Wagner T, Nilson JH. Expression of the glycoprotein hormone alpha-subunit gene in the placenta requires a functional cyclic AMP response element, whereas a different cis-acting element mediates pituitary-specific expression. Mol Cell Biol 9:5113–5122;1989.

5 Bradford AP, Conrad KE, Wasylyk C, Wasylyk B, Gutierrez-Hartmann A. Functional interaction of c-Ets-1 and GHF-1/Pit-1 mediates Ras activation of pituitary-specific gene expression: mapping of the essential c-Ets-1 domain. Mol Cell Biol 15:2849–2857;1995.

6 Bradford AP, Wasylyk C, Wasylyk B, Gutierrez-Hartmann A. Interaction of Ets-1 and the POU-homeodomain protein GHF-1/Pit-1 reconstitutes pituitary-specific gene expression. Mol Cell Biol 17:1065–1074;1997.

7 Denef C. Paracrine control of lactotrope proliferation and differentiation. Trends Endocrinol Metabol 14:188–195;2003.

8 Dohler KD, Von Zur MA, Dohler U. Pituitary luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin from birth to puberty in female and male rats. Acta Endocrinol (Copenh) 85:718–728;1977.

9 Ebling FJ, Cronin AS. The neurobiology of reproductive development. Neuroreport 11: R23-R33;2000.

10 Fink L, Seeger W, Ermert L, Hanze J, Stahl U, Grimminger F, Kummer W, Bohle RM. Real-time quantitative RT-PCR after laser-assisted cell picking. Nat Med 4:1329–1333;1998.

11 Gordon DF, Lewis SR, Haugen BR, James RA, McDermott MT, Wood WM, Ridgway EC. Pit-1 and GATA-2 interact and functionally cooperate to activate the thyrotropin beta-subunit promoter. J Biol Chem 272:24339–24347; 1997.

12 Gygi SP, Rochon Y, Franza BR, Aebersold R. Correlation between protein and mRNA abundance in yeast. Mol Cell Biol 19:1720–1730; 1999.

13 Hamernik DL, Keri RA, Clay CM, Clay JN, Sherman GB, Sawyer HR Jr, Nett TM, Nilson JH. Gonadotrope- and thyrotrope-specific expression of the human and bovine glycoprotein hormone alpha-subunit genes is regulated by distinct cis-acting elements. Mol Endocrinol 6: 1745–1755;1992.

14 Hauspie A, Seuntjens E, Vankelecom H, Denef C. Stimulation of combinatorial expression of prolactin and glycoprotein hormone alpha-subunit genes by gonadotropin-releasing hormone and estradiol-17beta in single rat pituitary cells during aggregate cell culture. Endocrinology 144:388–399;2003.

15 Héritier AG, Dubois PM. Re-evaluation of gonadotropin-releasing hormone (GnRH) action on pituitary cell differentiation with special regard to its effect on LH and TSH cell types. J Neuroendocrinol 6:33–37;1994.

16 Howard PW, Maurer RA. A composite Ets/Pit-1 binding site in the prolactin gene can mediate transcriptional responses to multiple signal transduction pathways. J Biol Chem 270: 20930–20936;1995.

17 Kendall SK, Saunders TL, Jin L, Lloyd RV, Glode LM, Nett TM, Keri RA, Nilson JH, Camper SA. Targeted ablation of pituitary gonadotropes in transgenic mice. Mol Endocrinol 5:2025–2036;1991.

18 Lipkin SM, Naar AM, Kalla KA, Sack RA, Rosenfeld MG. Identification of a novel zinc-finger protein binding a conserved element critical for Pit-1-dependent growth hormone gene expression. Genes Dev 7:1674–1687; 1993.

19 Meijer JC, Trudeau VL, Colenbrander B, Poot P, Erkens JH, Van de Wiel DF. Prolactin in the developing pig. Biol Reprod 39:264–269;1988.

20 Michael SD, Kaplan SB, Macmillan BT. Peripheral plasma concentrations of LH, FSH, prolactin and GH from birth to puberty in male and female mice. J Reprod Fertil 59:217–222; 1980.

21 Molesh DA, Hall JM. Quantitative analysis of CD34+ stem cells using RT-PCR on whole cells. PCR Methods Appl 3:278–284;1994.

22 Orlando C, Pinzani P, Pazzagli M. Developments in quantitative PCR. Clin Chem Lab Med 36:255–269;1998.

23 Pessin JE, Okada S. Insulin and EFG receptors integrate the Ras and Rap sigaling pathways. Endocr J 46:S11-S16;1999.

24 Risma KA, Clay CM, Nett TM, Wagner T, Yun J, Nilson JH. Targeted overexpression of luteinizing hormone in transgenic mice leads to infertility, polycystic ovaries, and ovarian tumors. Proc Natl Acad Sci USA 92:1322–1326; 1995.

25 Roudbaraki M, Lorsignol A, Langouche L, Callewaert G, Vankelecom H, Denef C. Target cells of gamma3-melanocyte-stimulating hormone detected through intracellular Ca^{2+} responses in immature rat pituitary constitute a fraction of all main pituitary cell types, but mostly express multiple hormone phenotypes at the messenger ribonucleic acid level. Refractoriness to melanocortin-3 receptor blockade in the lacto-somatotroph lineage. Endocrinology 140:4874–4885;1999.

26 Savoie S, Bourcl B, Hamel R, Buithieu M, Jequier JC, Bertrand J, Saez JM, Collu R, Ducharme JR. Circulating LH, FSH, prolactin, testosterone, delta 4-androstenedione, dehydroepiandrosterone sulfate and cortisol levels in the fetus in late gestation and in newborn male and female lambs. Horm Res 15:122–132;1981.

27 Schaufele F, West BL, Baxter JD. Synergistic activation of the rat growth hormone promoter by Pit-1 and the thyroid-hormone receptor. Mol Endocrinol 6:656–665;1992.

28 Scully KM, Jacobson EM, Jepsen K, Lunyak V, Viadiu H, Carrière C, Rose DW, Hooshmand F, Aggarwal AK, Rosenfeld MG. Allosteric effects of Pit-1 DNA sites on long-term repression in cell type specification. Science 290: 1127–1131;2000.

29 Scully KM, Rosenfeld MG. Pituitary development: regulatory codes in mammalian organogenesis. Science 295:2231–2235;2002.

30 Seuntjens E, Hauspie A, Vankelecom H, Denef C. Ontogeny of plurihormonal cells in the anterior pituitary of the mouse, as studied by means of hormone mRNA detection in single cells. J Neuroendocrinol 14:611–619;2002.

31 Seuntjens E, Vankelecom H, Quaegebeur A, Vande Vijver V, Denef C. Targeted ablation of gonadotrophs in transgenic mice affects embryonic development of lactotrophs. Mol Cell Endocrinol 150:129–139;1999.

32 Simmons DM, Voss JW, Ingraham HA, Holloway JM, Broide RS, Rosenfeld MG, Swanson LW. Pituitary cell phenotypes involve cell-specific Pit-1 mRNA translation and synergistic interactions with other classes of transcription factors. Genes Dev 4:695–711;1990.

33 Tilemans D, Andries M, Denef C. Luteinizing hormone-releasing hormone and neuropeptide Y influence deoxyribonucleic acid replication in three anterior pituitary cell types. Evidence for mediation by growth factors released from gonadotrophs. Endocrinology 130:882–894; 1992.

34 Van Bael A, Vande Vijver V, Devreese B, Van Beeumen J, Denef C. N-terminal 10- and 12-kDa POMC fragments stimulate differentiation of lactotrophs. Peptides 17:1219–1228; 1996.

35 Verbeke G, Molenberghs G. Linear mixed models in practice: A SAS-oriented approach; in Statistics (ed 126). New York, Springer, 1997.

36 Xu L, Lavinsky RM, Dasen JS, Flynn SE, McInerney EM, Mullen TM, Heinzel T, Szeto D, Korzus E, Kurokawa R, Aggarwal AK, Rose DW, Glass CK, Rosenfeld MG. Signal-specific co-activator domain requirements for Pit-1 activation. Nature 395:301–306;1998.

Subject Index Vol. 10, 2003

Journal of
Biomedical Science

ABC transporter
Multidrug resistance, MRP7, Genomic organization, Promoter analysis 98

Action potential
Atrial fibrillation, Triggered activity 535

Active transport
Erythrocyte, Na$^+$ pump, Ouabain binding, Sepsis 389

Acute lung injury
Adenosine, Adenosine triphosphate, Leukocytes 725

Adenoma
Polymeric immunoglobulin receptor, 3′UTR, RNA stability, mRNA differential display, Colon cancer, Neoplastic cell transformation 792

Adenosine
Adenosine triphosphate, Acute lung injury, Leukocytes 725

Adenosine triphosphate
Adenosine, Acute lung injury, Leukocytes 725

Adherens junction
crumbs, Cell polarity, Differentiation, photoreceptor, Rhabdomere 766

Adhesion
Elk-1, HIF-1, MEK, p42/p44 ERK, PAI-1 738

Adrenergic control
Sino-atrial node spontaneous discharge, Norepinephrine, Cesium, Barium, High [K$^+$]$_o$ indapamide, Hyperpolarization activated current I$_f$, Slow inward current I$_{Ca,L}$, Delayed rectifier current I$_K$ 179

α$_2$-Adrenoceptors
Morphine, Thrombosis, Cyclic AMP, Na$^+$/H$^+$ exchanger 292

AHPN
Apoptosis, Differentiation, CD437, DNA adduct 44

AIDS
Anti-Vpu antibody, IgG subclasses, *vpu* gene, Viral load, Total IgG, Disease progression, Highly active antiretroviral therapy 266

Alcohol dehydrogenase
Binding affinity, Docking, Substrate binding pocket, Zinc ion 302

Allergen
Bermuda grass pollen, Purification, Carbohydrate 111

Alzheimer's disease
β-Amyloid, Apoptosis, Reactive oxygen species 379

Amlodipine
Calcium channel blockers, Atherosclerosis, Cholesterol, Lipid peroxidation, Superoxide dismutase, Catalase 65

β-Amyloid
Alzheimer's disease, Apoptosis, Reactive oxygen species 379

Angiogenesis
ets-1, Uterine endometrium 320

Angiogenesis
Limbal fibroblast, Endothelial cell, Tissue inhibitor of metalloproteinase-3, Recombinant adenovirus 526

Anti-E2/NS1 prevalence
Chronic hepatitis, E2/NS1, Hepatitis C virus, Infection, Neutralizing antibodies 276

Antigen presentation
Mesenchymal stem cells, T lymphocytes, Hematopoietic interactions, Immune function 228

Antioxidants
Ascorbate, Ascorbyl palmitate, Carotid body, Cat, Ascorbate synthesis, Cellular membranes, HPLC, Hypoxia, Cerebral cortex 193

Antisense oligonucleotide
Leptin receptor, Sympathetic vasomotor tone, Food intake, Hypothalamic paraventricular nucleus, Systemic arterial pressure 367

Antitumor effect
In vivo electroporation, Interleukin-12, Cytokine gene therapy, 38C13 B-cell lymphoma, CT-26 colon adenocarcinoma, B16F1 melanoma 73

Antiviral therapy
Hepatitis B virus, Hepatitis C virus, ELISPOT assay, Tetramer assay, Limiting dilution analysis 120

Anti-Vpu antibody
IgG subclasses, *vpu* gene, Viral load, Total IgG, Disease progression, Highly active antiretroviral therapy, AIDS 266

Apnoea
Feline breathing control, Nodose ganglion, Serotonin, Vagotomy 718

Apolipoprotein-E-deficient mice
Atherosclerosis, Collagen, Iron, Matrix metalloproteinases, Plaque stability 510

Apolipoprotein E
Nephropathy, Type 2 diabetes, Glomerular filtration rate 260

Apolipoprotein E
Oxidized low-density lipoprotein, Triglyceride, Low-density lipoprotein size, Atherosclerosis 345

Apoptosis
Alzheimer's disease, β-Amyloid, Reactive oxygen species 379

Apoptosis
Differentiation, CD437, AHPN, DNA adduct 44

Apoptosis
Neutrophil, Protease, Phagocytosis, Lung injury 746

Applied lectins and carbohydrate specificities
Disaccharide units, Glycoproteins 676

Ascorbate
Antioxidants, Ascorbyl palmitate, Carotid body, Cat, Ascorbate synthesis, Cellular membranes, HPLC, Hypoxia, Cerebral cortex 193

Ascorbate synthesis
Antioxidants, Ascorbate, Ascorbyl palmitate, Carotid body, Cat, Cellular membranes, HPLC, Hypoxia, Cerebral cortex 193

Ascorbyl palmitate
Antioxidants, Ascorbate, Carotid body, Cat, Ascorbate synthesis, Cellular membranes, HPLC, Hypoxia, Cerebral cortex 193

Atherogenesis
Cytomegalovirus, NADPH oxidase, Smooth muscle, Coronary artery, Oxidative stress 505

Atherosclerosis
Amlodipine, Calcium channel blockers, Cholesterol, Lipid peroxidation, Superoxide dismutase, Catalase 65

Atherosclerosis
Apolipoprotein-E-deficient mice, Collagen, Iron, Matrix metallo-proteinases, Plaque stability 510
Atherosclerosis
Oxidized low-density lipoprotein, Apolipoprotein E, Triglyceride, Low-density lipoprotein size 345
Atrial fibrillation
Action potential, Triggered activity 535
Autoantibodies
Autoimmunity, Cross-reactivity DNA 544
Autoimmunity
Autoantibodies, Cross-reactivity DNA 544

B^{0+} system
Glycine transport, Transport proteins, Neurotransmitters, Vesicular inhibitory amino acid transporter 30
B16F1 melanoma
In vivo electroporation, Interleukin-12, Cytokine gene therapy, Antitumor effect, 38C13 B-cell lymphoma, CT-26 colon adenocarcinoma 73
Barium
Sino-atrial node spontaneous discharge, Adrenergic control, Norepinephrine, Cesium, High $[K^+]_o$ indapamide, Hyperpolarization activated current I_f, Slow inward current $I_{Ca,L}$, Delayed rectifier current I_K 179
Bermuda grass pollen
Allergen, Purification, Carbohydrate 111
Binding affinity
Alcohol dehydrogenase, Docking, Substrate binding pocket, Zinc ion 302
Bitter gourd
Peroxisome proliferator-activated receptor α, Stable transfection, Hypolipidemic 782
Breast cancer
HER-2 antibody, Immunolipoplex, Targeted gene delivery 337

38C13 B-cell lymphoma
In vivo electroporation, Interleukin-12, Cytokine gene therapy, Antitumor effect, CT-26 colon adenocarcinoma, B16F1 melanoma 73
Calcium-binding protein
S100P, S100, Retinoic acid, Differential display, Gastric cancer 313
Calcium channel blockers
Amlodipine, Atherosclerosis, Cholesterol, Lipid peroxidation, Superoxide dismutase, Catalase 65
Calcium signaling
HIV-1 enteropathy, Virotoxin, Protein kinase C, Intestinal cell line, GPR15/Bob coreceptor, Glycolipid, Lipid raft 156
Calisthenics
Wai tan kung, Exercise, Heart rate variability, Vagal modulation, Sympathetic modulation, Sympathetic reserve 697
Cancer
Gene therapy, Immunotherapy, Vaccine 37
Carbohydrate
Bermuda grass pollen, Allergen, Purification 111
Cardiovascular regulation
Nitric oxide synthase I, II, III, Rostral ventrolateral medulla, Sympathetic premotor neurons 285
Carotid body
Antioxidants, Ascorbate, Ascorbyl palmitate, Cat, Ascorbate synthesis, Cellular membranes, HPLC, Hypoxia, Cerebral cortex 193

Catalase
Amlodipine, Calcium channel blockers, Atherosclerosis, Cholesterol, Lipid peroxidation, Superoxide dismutase 65
Catalytic RNA
Hammerheads, Hairpins, Introns 457
Cat
Antioxidants, Ascorbate, Ascorbyl palmitate, Carotid body, Ascorbate synthesis, Cellular membranes, HPLC, Hypoxia, Cerebral cortex 193
CD437
Apoptosis, Differentiation, AHPN, DNA adduct 44
Cell fusions
Transforming growth factor beta, Signal transduction, Complementation, Colon 253
Cell polarity
crumbs, Adherens junction, Differentiation, photoreceptor, Rhabdomere 766
Cell proliferation
Mitogen-activated protein kinase, MEK, Tyrosine kinase, Protein kinase C, Vascular smooth muscle cell 208
Cellular membranes
Antioxidants, Ascorbate, Ascorbyl palmitate, Carotid body, Cat, Ascorbate synthesis, HPLC, Hypoxia, Cerebral cortex 193
Cerebral cortex
Antioxidants, Ascorbate, Ascorbyl palmitate, Carotid body, Cat, Ascorbate synthesis, Cellular membranes, HPLC, Hypoxia 193
Cerebral hemorrhage
Learning and memory, Immunohistochemistry, c-Fos, Cognitive function, Long-term potentiation, Patch clamp, Functional magnetic resonance image, Hippocampus, Striatum 14
Cesium
Sino-atrial node spontaneous discharge, Adrenergic control, Norepinephrine, Barium, High $[K^+]_o$ indapamide, Hyperpolarization activated current I_f, Slow inward current $I_{Ca,L}$, Delayed rectifier current I_K 179
c-Fos
Learning and memory, Immunohistochemistry, Cognitive function, Long-term potentiation, Patch clamp, Functional magnetic resonance image, Cerebral hemorrhage, Hippocampus, Striatum 14
Cholesterol
Amlodipine, Calcium channel blockers, Atherosclerosis, Lipid peroxidation, Superoxide dismutase, Catalase 65
Chronic hepatitis
Anti-E2/NS1 prevalence, E2/NS1, Hepatitis C virus, Infection, Neutralizing antibodies 276
Citrate synthase
Insulin resistance, Type 2 diabetes, Glycogen, Skeletal muscle 689
Coagulation factor IX protein
Randomness, Variants 451
Codon 72
p53, Prostate cancer 430
Cognitive function
Learning and memory, Immunohistochemistry, c-Fos, Long-term potentiation, Patch clamp, Functional magnetic resonance image, Cerebral hemorrhage, Hippocampus, Striatum 14
Collagen
Apolipoprotein-E-deficient mice, Atherosclerosis, Iron, Matrix metalloproteinases, Plaque stability 510
Collagen
Fibrosis, Malondialdehyde, 8-Hydroxy-2′-deoxyguanosine, Transforming growth factor-β1, Mitochondrial electron transport chain 170

Fibrosis
Malondialdehyde, 8-Hydroxy-2′-deoxyguanosine, Transforming growth factor-β1, Collagen, Mitochondrial electron transport chain 170

Fibrosis
Spironolactone, Hypertension, Myocardial remodeling, Stereology 50

Filtration coefficient
Ischemia, Lung injury, Nitric oxide, Reperfusion 58

FLIP1
LKB1, NF-κB inhibitor, Peutz-Jeghers syndrome, Protein kinase, Tumor-suppressor gene 242

Food intake
Leptin receptor, Sympathetic vasomotor tone, Antisense oligonu-cleotide, Hypothalamic paraventricular nucleus, Systemic arterial pressure 367

Functional magnetic resonance image
Learning and memory, Immunohistochemistry, c-Fos, Cognitive function, Long-term potentiation, Patch clamp, Cerebral hemor-rhage, Hippocampus, Striatum 14

Gammaherpesvirus
Human herpesvirus 8, Viral signalling ligand, Kaposi's sarcoma, Primary effusion lymphoma, Multicentric Castleman's disease 475

Gastric cancer
S100P, S100, Calcium-binding protein, Retinoic acid, Differential display 313

Gene delivery methods
Gene therapy, Mesenchymal stem cells, Microinjection 328

Gene repair
Head injury, Immediate-early genes, Mutagenesis, Neuroregenera-tion, Oxidative stress, Plasticity, Signal transduction, Stroke, Tran-scription 4

Gene therapy
Gene delivery methods, Mesenchymal stem cells, Microinjection 328

Gene therapy
Immunotherapy, Cancer, Vaccine 37

Genetic diversity
HIV-1, Vertical transmission, *nef* 436

Genetics
Severe acute respiratory syndrome, Coronavirus, Taxonomy, Origin, RNA, Viral proteins, Mutations, Pathogenesis, Replication, Drug Targets, Vaccines 664

Genomic organization
Multidrug resistance, ABC transporter, MRP7, Promoter analysis 98

Glomerular filtration rate
Apolipoprotein E, Nephropathy, Type 2 diabetes 260

Glutamate receptor
P19 cells, Kainic acid, Hypoxia, Nerve growth factor 199

Glycine N-methyltransferase
Recombinant protein, Monoclonal antibody, Epitope mapping, Hepatocellular carcinoma 87

Glycine transport
Transport proteins, Neurotransmitters, B^{0+} system, Vesicular inhibitory amino acid transporter 30

Glycogen
Insulin resistance, Citrate synthase, Type 2 diabetes, Skeletal muscle 689

Glycolipid
HIV-1 enteropathy, Virotoxin, Calcium signaling, Protein kinase C, Intestinal cell line, GPR15/Bob coreceptor, Lipid raft 156

Glycoproteins
Disaccharide units, Applied lectins and carbohydrate specificities 676

Gonadotroph
Transgenic mouse, Targeted cell ablation, Diphtheria toxin A, Pituitary, Messenger RNA, Quantitative RT-PCR, Prolactin, Growth hormone, Luteinizing hormone-β 805

GPIa polymorphisms
Collagen receptors, PFA-100™, Platelet adhesion, Platelet inhibi-tors 731

GPR15/Bob coreceptor
HIV-1 enteropathy, Virotoxin, Calcium signaling, Protein kinase C, Intestinal cell line, Glycolipid, Lipid raft 156

Growth hormone
Transgenic mouse, Targeted cell ablation, Diphtheria toxin A, Pituitary, Messenger RNA, Quantitative RT-PCR, Gonadotroph, Prolactin, Luteinizing hormone-β 805

Hairpins
Catalytic RNA, Hairpins, Introns 457

Hammerheads
Catalytic RNA, Hairpins, Introns 457

Head injury
Gene repair, Immediate-early genes, Mutagenesis, Neuroregenera-tion, Oxidative stress, Plasticity, Signal transduction, Stroke, Tran-scription 4

Heart rate variability
Wai tan kung, Calisthenics, Exercise, Vagal modulation, Sympa-thetic modulation, Sympathetic reserve 697

Hematopoietic interactions
Mesenchymal stem cells, Antigen presentation, T lymphocytes, Immune function 228

Hemophagocytic syndrome
Epstein-Barr virus, LMP-1, T cell lymphoma, Tumor necrosis factor-α 146

Hepatic stellate cells
Myofibroblast, Kupffer cells, Interleukin-1, Matrix metalloprotei-nase, p38 757

Hepatitis B virus
Antiviral therapy, Hepatitis C virus, ELISPOT assay, Tetramer assay, Limiting dilution analysis 120

Hepatitis C virus
Anti-E2/NS1 prevalence, Chronic hepatitis, E2/NS1, Infection, Neutralizing antibodies 276

Hepatitis C virus
Antiviral therapy, Hepatitis B virus, ELISPOT assay, Tetramer assay, Limiting dilution analysis 120

Hepatocellular carcinoma
Glycine N-methyltransferase, Recombinant protein, Monoclonal antibody, Epitope mapping 87

HER-2 antibody
Immunolipoplex, Targeted gene delivery, Breast cancer 337

HIF-1
Adhesion, Elk-1, MEK, p42/p44 ERK, PAI-1 738

High $[K^+]_o$ indapamide
Sino-atrial node spontaneous discharge, Adrenergic control, Norepinephrine, Cesium, Barium, Hyperpolarization activated current I_f, Slow inward current $I_{Ca,L}$, Delayed rectifier current I_K 179

Highly active antiretroviral therapy
Anti-Vpu antibody, IgG subclasses, *vpu* gene, Viral load, Total IgG, Disease progression, AIDS 266

Limiting dilution analysis
Antiviral therapy, Hepatitis B virus, Hepatitis C virus, ELISPOT assay, Tetramer assay 120

Lipid peroxidation
Amlodipine, Calcium channel blockers, Atherosclerosis, Cholesterol, Superoxide dismutase, Catalase 65

Lipid raft
HIV-1 enteropathy, Virotoxin, Calcium signaling, Protein kinase C, Intestinal cell line, GPR15/Bob coreceptor, Glycolipid 156

Lipopolysaccharides
Nuclear factor κB, Oxygen free radicals, Sepsis, Tissue factor 518

Lipoteichoic acid
Inducible nitric oxide synthase, Nitric oxide, Protein kinase C, NF-κB, RAW 264.7 macrophages 136

Liver
Lysophosphatidic acid, Myosin, Rho-associated kinase, Phosphorylation, Contraction, Stress-activated protein kinase 352

LKB1
FLIP1, NF-κB inhibitor, Peutz-Jeghers syndrome, Protein kinase, Tumor-suppressor gene 242

LMP-1
Epstein-Barr virus, T cell lymphoma, Hemophagocytic syndrome, Tumor necrosis factor-α 146

Long-term potentiation
Learning and memory, Immunohistochemistry, c-Fos, Cognitive function, Patch clamp, Functional magnetic resonance image, Cerebral hemorrhage, Hippocampus, Striatum 14

Low-density lipoprotein size
Oxidized low-density lipoprotein, Apolipoprotein E, Triglyceride, Atherosclerosis 345

Lung injury
Apoptosis, Neutrophil, Protease, Phagocytosis 746

Lung injury
Filtration coefficient, Ischemia, Nitric oxide, Reperfusion 58

Luteinizing hormone-β
Transgenic mouse, Targeted cell ablation, Diphtheria toxin A, Pituitary, Messenger RNA, Quantitative RT-PCR, Gonadotroph, Prolactin, Growth hormone 805

Lysophosphatidic acid
Myosin, Liver, Rho-associated kinase, Phosphorylation, Contraction, Stress-activated protein kinase 352

Macrophage
Inducible nitric oxide synthase, Statins, HMG-CoA reductase, NF-κB, STAT1 396

Malondialdehyde
Fibrosis, 8-Hydroxy-2'-deoxyguanosine, Transforming growth factor-β1, Collagen, Mitochondrial electron transport chain 170

Matrin 3
DNA-binding protein, Medulloblastoma, p21, Nuclear protein 406

Matrix metalloproteinase
Hepatic stellate cells, Myofibroblast, Kupffer cells, Interleukin-1, p38 757

Matrix metalloproteinases
Apolipoprotein-E-deficient mice, Atherosclerosis, Collagen, Iron, Plaque stability 510

Medulloblastoma
DNA-binding protein, Matrin 3, p21, Nuclear protein 406

MEK
Adhesion, Elk-1, HIF-1, p42/p44 ERK, PAI-1 738

MEK
Cell proliferation, Mitogen-activated protein kinase, Tyrosine kinase, Protein kinase C, Vascular smooth muscle cell 208

Membrane-associated tyrosine hydroxylase
Dopamine synthesis, Synaptic vesicles, Protein phosphorylation, Dopamine packaging 774

Membrane potential
Mitochondria, Nasopharyngeal carcinoma, Porphycene, Photodynamic therapy 418

Mesenchymal stem cells
Antigen presentation, T lymphocytes, Hematopoietic interactions, Immune function 228

Mesenchymal stem cells
Gene delivery methods, Gene therapy, Microinjection 328

Messenger RNA
Transgenic mouse, Targeted cell ablation, Diphtheria toxin A, Pituitary, Quantitative RT-PCR, Gonadotroph, Prolactin, Growth hormone, Luteinizing hormone-β 805

Metabolic ratio
Dextromethorphan, Dextrorphan, Pharmacokinetic parameters, Steady state 552

Microinjection
Gene delivery methods, Gene therapy, Mesenchymal stem cells 328

Mitochondrial electron transport chain
Fibrosis, Malondialdehyde, 8-Hydroxy-2'-deoxyguanosine, Transforming growth factor-β1, Collagen 170

Mitochondria
Membrane potential, Nasopharyngeal carcinoma, Porphycene, Photodynamic therapy 418

Mitogen-activated protein kinase
Cell proliferation, MEK, Tyrosine kinase, Protein kinase C, Vascular smooth muscle cell 208

Monoclonal antibody
Glycine N-methyltransferase, Recombinant protein, Epitope mapping, Hepatocellular carcinoma 87

Morphine
Thrombosis, α_2-Adrenoceptors, Cyclic AMP, Na^+/H^+ exchanger 292

mRNA differential display
Polymeric immunoglobulin receptor, 3'UTR, RNA stability, Colon cancer, Adenoma, Neoplastic cell transformation 792

MRP7
Multidrug resistance, ABC transporter, Genomic organization, Promoter analysis 98

Multicentric
Castleman's disease Human herpesvirus 8, Gammaherpesvirus, Viral signalling ligand, Kaposi's sarcoma, Primary effusion lymphoma 475

Multidrug resistance
ABC transporter, MRP7, Genomic organization, Promoter analysis 98

Multidrug resistance
Visible light, Phototherapy, Photosensitizer, Review 361

Mutagenesis
Gene repair, Head injury, Immediate-early genes, Neuroregeneration, Oxidative stress, Plasticity, Signal transduction, Stroke, Transcription 4

Mutations
Severe acute respiratory syndrome, Coronavirus, Taxonomy, Origin, RNA, Viral proteins, Genetics, Pathogenesis, Replication, Drug Targets, Vaccines 664

Myocardial remodeling
Spironolactone, Hypertension, Fibrosis, Stereology 50
Myofibroblast
Hepatic stellate cells, Kupffer cells, Interleukin-1, Matrix metallo-
proteinase, p38 757
Myosin
Lysophosphatidic acid, Liver, Rho-associated kinase, Phosphoryla-
tion, Contraction, Stress activated protein kinase 352

NADPH oxidase
Cytomegalovirus, Smooth muscle, Coronary artery, Oxidative
stress, Atherogenesis 505
Na^+/H^+ exchanger
Morphine, Thrombosis, α_2-Adrenoceptors, Cyclic AMP 292
Na^+ pump
Active transport, Erythrocyte, Ouabain binding, Sepsis 389
Nasopharyngeal carcinoma
DNA methylation, Epstein-Barr virus, Latent membrane protein
1, Signal transduction 490
Nasopharyngeal carcinoma
Mitochondria, Membrane potential, Porphycene, Photodynamic
therapy 418
nef
HIV-1, Vertical transmission, Genetic diversity 436
Neoplastic cell transformation
Polymeric immunoglobulin receptor, 3′UTR, RNA stability,
mRNA differential display, Colon cancer, Adenoma 792
Nephropathy
Apolipoprotein E, Type 2 diabetes, Glomerular filtration rate 260
Nerve growth factor
P19 cells, Glutamate receptor, Kainic acid, Hypoxia 199
Neuroregeneration
Gene repair, Head injury, Immediate-early genes, Mutagenesis,
Oxidative stress, Plasticity, Signal transduction, Stroke, Transcrip-
tion 4
Neurotransmitters
Glycine transport, Transport proteins, B^{0+} system, Vesicular inhib-
itory amino acid transporter 30
Neutralizing antibodies
Anti-E2/NS1 prevalence, Chronic hepatitis, E2/NS1, Hepatitis C
virus, Infection 276
Neutrophil
Apoptosis, Protease, Phagocytosis, Lung injury 746
NF-κB
Inducible nitric oxide synthase, Statins, HMG-CoA reductase,
STAT1, Macrophage 396
NF-κB inhibitor
FLIP1, LKB1, Peutz-Jeghers syndrome, Protein kinase, Tumor-
suppressor gene 242
NF-κB
Lipoteichoic acid, Inducible nitric oxide synthase, Nitric oxide,
Protein kinase C, RAW 264.7 macrophages 136
NF-κB
RanGTPase, Septic shock, Immune response 468
Nitric oxide
Filtration coefficient, Ischemia, Lung injury, Reperfusion 58
Nitric oxide
Lipoteichoic acid, Inducible nitric oxide synthase, Protein kinase
C, NF-κB, RAW 264.7 macrophages 136
Nitric oxide synthase I, II, III
Rostral ventrolateral medulla, Cardiovascular regulation, Sympa-
thetic premotor neurons 285

Nodose ganglion
Apnoea, Feline breathing control, Serotonin, Vagotomy 718
Norepinephrine
Sino-atrial node spontaneous discharge, Adrenergic control,
Cesium, Barium, High $[K^+]_o$ indapamide, Hyperpolarization acti-
vated current I_f, Slow inward current $I_{Ca,L}$, Delayed rectifier
current I_K 179
Nuclear factor κB
Lipopolysaccharides, Oxygen free radicals, Sepsis, Tissue factor
518
Nuclear protein
DNA-binding protein, Matrin 3, Medulloblastoma, p21 406

Origin
Severe acute respiratory syndrome, Coronavirus, Taxonomy,
RNA, Viral proteins, Genetics, Mutations, Pathogenesis, Replica-
tion, Drug Targets, Vaccines 664
Ouabain binding
Active transport, Erythrocyte, Na^+ pump, Sepsis 389
Oxidative stress
Cytomegalovirus, NADPH oxidase, Smooth muscle, Coronary
artery, Atherogenesis 505
Oxidative stress
Gene repair, Head injury, Immediate-early genes, Mutagenesis,
Neuroregeneration, Plasticity, Signal transduction, Stroke, Tran-
scription 4
Oxidized low-density lipoprotein
Apolipoprotein E, Triglyceride, Low-density lipoprotein size,
Atherosclerosis 345
Oxygen free radicals
Lipopolysaccharides, Nuclear factor κB, Sepsis, Tissue factor 518

P19 cells
Glutamate receptor, Kainic acid, Hypoxia, Nerve growth factor
199
p21
DNA-binding protein, Matrin 3, Medulloblastoma, Nuclear
protein 406
p21/WAF1
EGCG, p53, Fas/APO-1, Fas ligand, apoptosis 219
p38
Hepatic stellate cells, Myofibroblast, Kupffer cells, Interleu-
kin-1, Matrix metalloproteinase 757
p42/p44 ERK
Adhesion, Elk-1, HIF-1, MEK, PAI-1 738
p53
Codon 72, Prostate cancer 430
p53
EGCG, p21/WAF1, Fas/APO-1, Fas ligand, apoptosis 219
PAI-1
Adhesion, Elk-1, HIF-1, MEK, p42/p44 ERK 738
Patch clamp
Learning and memory, Immunohistochemistry, c-Fos, Cognitive
function, Long-term potentiation, Functional magnetic resonance
image, Cerebral hemorrhage, Hippocampus, Striatum 14
Pathogenesis
Severe acute respiratory syndrome, Coronavirus, Taxonomy,
Origin, RNA, Viral proteins, Genetics, Mutations, Replication,
Drug Targets, Vaccines 664
Peroxisome proliferator-activated receptor α
Stable transfection, Bitter gourd, Hypolipidemic 782

Peutz-Jeghers syndrome
    FLIP1, LKB1, NF-κB inhibitor, Protein kinase, Tumor-suppressor gene 242
PFA-100™
    Collagen receptors, GPIa polymorphisms, Platelet adhesion, Platelet inhibitors 731
Phagocytosis
    Apoptosis, Neutrophil, Protease, Lung injury 746
Pharmacokinetic parameters
    Dextromethorphan, Dextrorphan, Metabolic ratio, Steady state 552
Phosphorylation
    Lysophosphatidic acid, Myosin, Liver, Rho-associated kinase, Contraction, Stress-activated protein kinase 352
Photodynamic therapy
    Mitochondria, Membrane potential, Nasopharyngeal carcinoma, Porphycene 418
Photosensitizer
    Multidrug resistance, Visible light, Phototherapy, Review 361
Phototherapy
    Multidrug resistance, Visible light, Photosensitizer, Review 361
Phrenic nerve
    Hypoglossal nerve, Pulmonary C-fibers, Vagotomy, Hypercapnia, Rat 706
Pituitary
    Transgenic mouse, Targeted cell ablation, Diphtheria toxin A, Messenger RNA, Quantitative RT-PCR, Gonadotroph, Prolactin, Growth hormone, Luteinizing hormone-β 805
Plaque stability
    Apolipoprotein-E-deficient mice, Atherosclerosis, Collagen, Iron, Matrix metalloproteinases 510
Plasticity
    Gene repair, Head injury, Immediate-early genes, Mutagenesis, Neuroregeneration, Oxidative stress, Signal transduction, Stroke, Transcription 4
Platelet adhesion
    Collagen receptors, GPIa polymorphisms, PFA-100™, Platelet inhibitors 731
Platelet inhibitors
    Collagen receptors, GPIa polymorphisms, PFA-100™, Platelet adhesion 731
Polymeric immunoglobulin receptor
    3'UTR, RNA stability, mRNA differential display, Colon cancer, Adenoma, Neoplastic cell transformation 792
Porphycene
    Mitochondria, Membrane potential, Nasopharyngeal carcinoma, Photodynamic therapy 418
Primary effusion lymphoma
    Human herpesvirus 8, Gammaherpesvirus, Viral signalling ligand, Kaposi's sarcoma, Multicentric Castleman's disease 475
Prolactin
    Transgenic mouse, Targeted cell ablation, Diphtheria toxin A, Pituitary, Messenger RNA, Quantitative RT-PCR, Gonadotroph, Growth hormone, Luteinizing hormone-β 805
Promoter analysis
    Multidrug resistance, ABC transporter, MRP7, Genomic organization 98
Prostate cancer
    p53, Codon 72 430
Protease
    Apoptosis, Neutrophil, Phagocytosis, Lung injury 746

Protein kinase C
    Cell proliferation, Mitogen-activated protein kinase, MEK, Tyrosine kinase, Vascular smooth muscle cell 208
Protein kinase C
    HIV-1 enteropathy, Virotoxin, Calcium signaling, Intestinal cell line, GPR15/Bob coreceptor, Glycolipid, Lipid raft 156
Protein kinase C
    Lipoteichoic acid, Inducible nitric oxide synthase, Nitric oxide, NF-κB, RAW 264.7 macrophages 136
Protein kinase
    FLIP1, LKB1, NF-κB inhibitor, Peutz-Jeghers syndrome, Tumor-suppressor gene 242
Protein phosphorylation
    Dopamine synthesis, Membrane-associated tyrosine hydroxylase, Synaptic vesicles, Dopamine packaging 774
Pulmonary C-fibers
    Hypoglossal nerve, Phrenic nerve, Vagotomy, Hypercapnia, Rat 706
Purification
    Bermuda grass pollen, Allergen, Carbohydrate 111

Quantitative RT-PCR
    Transgenic mouse, Targeted cell ablation, Diphtheria toxin A, Pituitary, Messenger RNA, Gonadotroph, Prolactin, Growth hormone, Luteinizing hormone-β 805

Randomness
    Coagulation factor IX protein, Variants 451
RanGTPase
    Septic shock, NF-κB, Immune response 468
Rat
    Hypoglossal nerve, Phrenic nerve, Recurrent laryngeal nerve, Pulmonary C-fibers, Vagotomy, Hypercapnia 706
RAW 264.7 macrophages
    Lipoteichoic acid, Inducible nitric oxide synthase, Nitric oxide, Protein kinase C, NF-κB 136
Reactive oxygen species
    Alzheimer's disease, β-Amyloid, Apoptosis 379
Recombinant adenovirus
    Limbal fibroblast, Endothelial cell, Angiogenesis, Tissue inhibitor of metalloproteinase-3 526
Recombinant protein
    Glycine N-methyltransferase, Monoclonal antibody, Epitope mapping, Hepatocellular carcinoma 87
Reperfusion
    Filtration coefficient, Ischemia, Lung injury, Nitric oxide 58
Replication
    Severe acute respiratory syndrome, Coronavirus, Taxonomy, Origin, RNA, Viral proteins, Genetics, Mutations, Pathogenesis, Drug Targets, Vaccines 664
Retinoic acid
    S100P, S100, Calcium-binding protein, Differential display, Gastric cancer 313
Review
    Multidrug resistance, Visible light, Phototherapy, Photosensitizer 361
Rhabdomere
    crumbs, Cell polarity, Adherens junction, Differentiation, photoreceptor 766
Rho-associated kinase
    Lysophosphatidic acid, Myosin, Liver, Phosphorylation, Contraction, Stress-activated protein kinase 352

RNA
   Severe acute respiratory syndrome, Coronavirus, Taxonomy,
   Origin, Viral proteins, Genetics, Mutations, Pathogenesis, Replica-
   tion, Drug Targets, Vaccines 664
RNA stability
   Polymeric immunoglobulin receptor, 3'UTR, mRNA differential
   display, Colon cancer, Adenoma, Neoplastic cell transformation
   792
Rostral ventrolateral medulla
   Nitric oxide synthase I, II, III, Cardiovascular regulation, Sympa-
   thetic premotor neurons 285

S100P
   S100, Calcium-binding protein, Retinoic acid, Differential display,
   Gastric cancer 313
S100
   S100P, Calcium-binding protein, Retinoic acid, Differential
   display, Gastric cancer 313
Sepsis
   Active transport, Erythrocyte, Na$^+$ pump, Ouabain binding 389
Sepsis
   Lipopolysaccharides, Nuclear factor κB, Oxygen free radicals,
   Tissue factor 518
Septic shock
   RanGTPase, NF-κB, Immune response 468
Serotonin
   Apnoea, Feline breathing control, Nodose ganglion, Vagotomy 718
Severe acute respiratory syndrome
   Coronavirus, Taxonomy, Origin, RNA, Viral proteins, Genetics,
   Mutations, Pathogenesis, Replication, Drug Targets, Vaccines 664
Signal transduction
   DNA methylation, Epstein-Barr virus, Latent membrane protein
   1, Nasopharyngeal carcinoma 490
Signal transduction
   Gene repair, Head injury, Immediate-early genes, Mutagenesis,
   Neuroregeneration, Oxidative stress, Plasticity, Stroke, Transcrip-
   tion 4
Signal transduction
   Transforming growth factor beta, Cell fusions, Complementation,
   Colon 253
Sino-atrial node spontaneous discharge
   Adrenergic control, Norepinephrine, Cesium, Barium, High [K$^+$]$_o$
   indapamide, Hyperpolarization activated current I$_f$, Slow inward
   current I$_{Ca,L}$, Delayed rectifier current I$_K$ 179
Skeletal muscle
   Insulin resistance, Citrate synthase, Type 2 diabetes, Glycogen 689
Slow inward current I$_{Ca,L}$
   Sino-atrial node spontaneous discharge, Adrenergic control,
   Norepinephrine, Cesium, Barium, High [K$^+$]$_o$ indapamide, Hyper-
   polarization activated current I$_f$, Delayed rectifier current I$_K$ 179
Smooth muscle
   Cytomegalovirus, NADPH oxidase, Coronary artery, Oxidative
   stress, Atherogenesis 505
Spironolactone
   Hypertension, Myocardial remodeling, Fibrosis, Stereology 50
Stable transfection
   Peroxisome proliferator-activated receptor α, Bitter gourd, Hypo-
   lipidemic 782
STAT1
   Inducible nitric oxide synthase, Statins, HMG-CoA reductase,
   NF-κB, Macrophage 396

Statins
   Inducible nitric oxide synthase, HMG-CoA reductase, NF-κB,
   STAT1, Macrophage 396
Steady state
   Dextromethorphan, Dextrorphan, Metabolic ratio, Pharmaco-
   kinetic parameters 552
Stereology
   Spironolactone, Hypertension, Myocardial remodeling, Fibrosis
   50
Stress-activated protein kinase
   Lysophosphatidic acid, Myosin, Liver, Rho-associated kinase,
   Phosphorylation, Contraction, Stress-activated protein kinase 352
Striatum
   Learning and memory, Immunohistochemistry, c-Fos, Cognitive
   function, Long-term potentiation, Patch clamp, Functional
   magnetic resonance image, Cerebral hemorrhage, Hippocampus
   14
Stroke
   Gene repair, Head injury, Immediate-early genes, Mutagenesis,
   Neuroregeneration, Oxidative stress, Plasticity, Signal transduc-
   tion, Transcription 4
Substrate binding pocket
   Alcohol dehydrogenase, Binding affinity, Docking, Zinc ion 302
Superoxide dismutase
   Amlodipine, Calcium channel blockers, Atherosclerosis, Choles-
   terol, Lipid peroxidation, Catalase 65
Sympathetic modulation
   Wai tan kung, Calisthenics, Exercise, Heart rate variability, Vagal
   modulation, Sympathetic reserve 697
Sympathetic premotor neurons
   Nitric oxide synthase I, II, III, Rostral ventrolateral medulla,
   Cardiovascular regulation 285
Sympathetic reserve
   Wai tan kung, Calisthenics, Exercise, Heart rate variability, Vagal
   modulation, Sympathetic modulation 697
Sympathetic vasomotor tone
   Leptin receptor, Food intake, Antisense oligonucleotide, Hypotha-
   lamic paraventricular nucleus, Systemic arterial pressure 367
Synaptic vesicles
   Dopamine synthesis, Membrane-associated tyrosine hydroxylase,
   Protein phosphorylation, Dopamine packaging 774
Systemic arterial pressure
   Leptin receptor, Sympathetic vasomotor tone, Food intake, Anti-
   sense oligonucleotide, Hypothalamic paraventricular nucleus 367

Targeted cell ablation
   Transgenic mouse, Diphtheria toxin A, Pituitary, Messenger RNA,
   Quantitative RT-PCR, Gonadotroph, Prolactin, Growth hormone,
   Luteinizing hormone-β 805
Targeted gene delivery
   HER-2 antibody, Immunolipoplex, Breast cancer 337
Taxonomy
   Severe acute respiratory syndrome, Coronavirus, Origin, RNA,
   Viral proteins, Genetics, Mutations, Pathogenesis, Replication,
   Drug Targets, Vaccines 664
T cell lymphoma
   Epstein-Barr virus, LMP-1, Hemophagocytic syndrome, Tumor
   necrosis factor-α 146
Tetramer assay
   Antiviral therapy, Hepatitis B virus, Hepatitis C virus, ELISPOT
   assay, Limiting dilution analysis 120
Thrombosis
   Morphine, α$_2$-Adrenoceptors, Cyclic AMP, Na$^+$/H$^+$ exchanger 292