Effects of Immunosuppressant Agents on Growth Hormone Release and Gene Expression

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THE immunosuppressant drug, FK506 (FK), acts through its binding protein, FK-binding protein (FKBP12) to suppress the function of T lymphocytes with relative selectivity [1–3]. Recent reports have indicated that FKBP12s are widely distributed in the central nervous system [4] including the hypothalamus, suggesting biological effects of FK on brain and neuroendocrine functions. Antoni *et al.* reported that FK stimulates ACTH secretion from AtT20 mouse pituitary corticotrope tumor cells [5]. We have also reported that FK stimulates both GH secretion and gene expression of hypothalamic GH-releasing hormone (GRH) in rats [6]. Although dual actions of FK to the hypothalamus and pituitary have been indicated in this paper, the intracellular signaling pathway has not yet been elucidated.

Other principal immunosuppressant drugs, cyclosporin A (CyA) and rapamycin (RP) bind to cyclophilin and FKBP12, respectively [2]. The major target of CyA-cyclophilin complex is a calcium and calmodulin dependent protein phosphatase, calcineurin (CaN), similar to FK, but RP does not affect CaN activity [2, 7], so that the signaling processes of these drugs are different. To investigate the intracellular signaling pathway in FK-induced GH release, these drugs were used in combination with FK in the present study. We also investigated whether FK stimulates not only

GH release, but also GH gene expression, because it is well established that the effects of FK are chiefly due to genomic action on target genes such as interleukin 2 in the T lymphocytes.

Materials and Methods

Primary culture of rat anterior pituitary cells was prepared as previously described [8]. Cultured pituitary cells were treated with CyA, RP and tumor growth factor- β (TGF- β) in the presence or absence of FK, and GH release in the culture medium was examined with a rat GH RIA kit supplied by NIDDK. Primary transcripts of rat GH gene were examined by the intron-specific polymerase chain reaction (PCR) method as follows. PCR primers were designed to amplify a 234 bp fragment from intron C to intron D of the rat GH gene [9]. The sense primer was 5'-TCACTAGCTAATGCACAGC-3' and the antisense primer was 5'-GTATAACCT-CAGGCTAGCG-3'. Sequence analysis of the PCR product with this pair of primers confirmed the rat GH gene. Total RNA was extracted from cultured rat anterior pituitary cells and then treated with DNase I to remove the residual contamination of genomic DNA. RNA was reverse transcribed into cDNA and PCR was performed as previously described [10]. PCR products were electrophoresed in agarose gel containing ethidium bromide and photographed. Statistical comparisons were made by one-way analysis of variance and Student's *t*-test, with $P < 0.05$ being considered significant.

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Results

Two-hour incubation with FK significantly increased GH release from cultured rat anterior pituitary cells in a dose-dependent manner (Fig. 1). The maximal effect was found at a concentration of 10 μ M. RP had no effect on GH release, whereas CyA moderately, but significantly, augmented GH release (Fig. 2). FK-induced GH release was

Fig. 1. Effects of FK on GH release from cultured rat anterior pituitary cells. The cells were incubated with FK for 2 h. Vertical lines represent the SEM. * $P < 0.05$ vs. control (c). Numbers in parentheses indicate the number of observations in each group.

Fig. 2. Effects of RP and CyA on GH release from cultured rat anterior pituitary cells. The cells were incubated with RP or CyA for 2 h. Vertical lines represent the SEM. ** $P < 0.01$ vs. control (c). Numbers in parentheses indicate the number of observations in each group.

significantly inhibited by coincubation with RP (Fig. 3). Tumor growth factor- β (TGF- β) did not affect basal GH release, but FK-induced GH release was significantly inhibited by coincubation with TGF- β (Fig. 4). Expression of the primary transcripts of

Fig. 3. Effects of RP on FK-induced GH release from cultured rat anterior pituitary cells. The cells were treated with RP in the presence of FK for 2 h. Vertical lines represent the SEM. Numbers in parentheses indicate the number of observations in each group.

Fig. 4. Effects of TGF on FK-induced GH release from cultured rat anterior pituitary cells. The cells were treated with TGF in the presence of FK for 2 h. Vertical lines represent the SEM. ** $P < 0.01$ vs. control (c). Numbers in parentheses indicate the number of observations in each group.

Fig. 5. Effects of FK on the expression of primary transcripts of rat GH gene in cultured rat anterior pituitary cells. The cells were incubated with FK or 0.1% ethanol for different time periods as indicated in the figure. The primary transcripts (224 bp) of the rat GH gene were detected by intron-specific PCR. FK stimulated the expression of primary transcripts from 30 min after the incubation. M: molecular marker.

rat GH gene was stimulated by FK in a time-dependent manner with the earliest effect occurring at 30 min (Fig. 5).

Discussion

The principal immunosuppressant drugs, FK, CyA and RP were introduced to the primary culture system of rat anterior pituitary cells to investigate the intracellular signaling pathway involved in FK-induced GH release in this study. FK significantly increased GH release in a dose-dependent manner as previously reported [6]. Similarly, CyA augmented GH release, although RP had no effect on GH release at the same concentration as FK and CyA. It has already been established that FK and CyA, when bound to their binding proteins such as FKBP12 and cyclophilin, inhibit CaN activity [2]. By contrast, RP does not inhibit CaN activity even though it binds to FKBP12 [2]. Taken together, it is likely that inhibition of CaN activity is primarily responsible for FK-induced GH release from rat somatotropes. Recent reports have indicated that FKBP12 regulates intracellular calcium ion channels [7] but this might play a minor role at least in GH secretion from somatotropes, since RP did not affect GH secretion. In our study,

Rp significantly inhibited FK-induced GH secretion, suggesting that RP competes with FK through their binding protein, FKBP12, in somatotropes.

Although TGF- β itself did not affect basal GH release, it significantly inhibited FK-induced GH release. Recent reports have shown a molecular interaction between FKBP12 and TGF- β receptor [11]. Our results also support this interaction on GH secretion from rat somatotropes. The interaction between FKBP12 and TGF receptors might play an additional role in the FKBP-CaN-GH signalling pathway. In the present study, FK stimulated expression of the primary transcripts of rat GH gene as well as GH release. GH mRNA content does not reflect the transcriptional activity of the GH gene, because a large amount of GH mRNA is stored in somatotropes. The primary transcripts of the GH gene (pre-mRNA) are located in the nuclei of somatotropes and rapidly released into the cytoplasm after splicing, so that the pre-mRNA of the GH gene is also comparable to its transcriptional activity. Our results suggest that the FK-FKBP complex stimulates the transcriptional activity of the rat GH gene. Further studies are required to elucidate whether the complex directly binds to a *cis*-element of the GH gene or affects *trans*-elements in somatotropes.

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