PERIFUSION SYSTEM: ITS USE IN THE STUDY OF THE NEUROENDOCRINE CONTROL OF HUMAN PITUITARY TUMORAL CELLS

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Regulation of hormone release involves both long term and short term controls, which depends upon which receptor is activated (nuclear or membrane) and upon which stimulating agent is used. When long term regulation is involved, protein synthesis is the major event determining the observed changes in hormone release. When short term regulation is involved, membrane transducing mechanisms are implicated and variations in ion fluxes, adenylate cyclase, phospholipase C, D or A2 activities are immediately followed by changes in hormone release. One same agent may have both long term and short term effects on hormone release as rapid changes in secretory rate, if they last, will end up in changes in synthesis rate.

However, in vitro, short and long term regulations need different approaches. Primary culture is well adapted to long term secretory regulation whereas short term regulations need a dynamic approach and the perifusion technique is particularly useful in this case. Indeed, it bathes the tissues in continually fresh medium, thereby eliminating feedback or waste product effects. The tissues can be exposed to various conditions without the artifacts arising from physical manipulations and finally, the time course of any effects produced may be observed. In particular, small changes in release rates which would be blunted in standard culture by the relatively large amount of basal release, will be easily detected.

We have used the perifusion system to study the neuroendocrine control of human pituitary tumoral cells, in order to further understand the dysregulation leading to the hypersecretory state of these tumors. Human pituitary tumors are of different types, secreting one or several hormones and nonsecreting. Among the secreting adenomas, we have focused our attention on the prolactinomas, secreting prolactin (PRL), and on the somatotropic adenomas secreting growth hormone (GH). Not only the perifusion system allowed us to study what could be called the "conventional" PRL and GH release regulation by hypothalamic neuropeptides, it also allowed us to observe differences in the regulation between adenomas of the same cell type and to establish a relationship between the cell response and the number of receptors for a given neuropeptide. Furthermore, we could demonstrate that normal and tumoral pituitary cells produce and release neuropeptides which may interfere with the secretions of pituitary hormones.

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Key Words: perifusion, pituitary adenomas, hormone regulation.
MATERIAL AND METHODS

Pituitary Tissue
Pituitary adenomas (PRL- and GH-secreting ones) were collected in the operating room after transsphenoidal adenomectomy. Human normal pituitaries were obtained 4 to 8 h after death from subjects (range 20 to 80 years old) with no evidence of neuroendocrine disorder.

Diagnosis before surgery was established on clinical, biological and radiological criteria. Morphological and immunocytochemical studies confirmed the diagnosis. All prolactinomas were associated with plasma PRL levels above 19 mg/l and somatotropic adenomas with plasma GH levels above 5 mg/l.

Perifusion
Fragments (15 mg) of pituitary tissue collected post-mortem or mechanically dispersed cells (1 x 10^6 cells) from pituitary adenomas were perifused in an isolated chamber at 37°C containing 0.4 g Bio-Gel P2. The perifusion medium contained 118 mmol/L NaCl, 4.7 mmol/L KCl, 1.18 mmol/L KH2PO4, 2.5 mmol/L CaCl2, 25 mmol/L NaHCO3, 1.18 mmol/L MgSO4 and 14 mmol/L glucose supplemented with 5 g/L BSA and a mixture of aminoacids. The medium was constantly gassed with CO2 - O2 (5-95%). Fractions (1 ml) were collected every 2 min and immediately frozen at -20°C until hormone assays.

Dose-dependencies were run as follows: after 90 min preincubation, successive 20-min pulses with increasing test substance concentrations were applied to the perifusion chamber. Two successive pulses were separated by 45-min perifusion with control medium. In all experiments, a perifusion chamber was run with control medium alone in order to assess basal hormone release. When maximal cell response was studied, two successive 20 min pulses with 10^-6 M of the test substance were run, separated by 45-min perifusion with control medium. In all experiments, the last 15 min consisted of a pulse with 47 mM KCl in order to depolarize the cells and then verify the viability of the cells and the intracellular hormone content.

Hormone Assays
PRL and GH were assayed by RIA, using kits obtained from Pharmacia (Uppsala, Sweden) Somatostatin (SRIH) was assayed by RIA using an anti-SRIH purchased from Amersham (Les Ulis, France); the procedure was that already described (Joubert et al., 1989).

RESULTS AND DISCUSSION

PRL and GH Release Regulation
As shown in Figure 1, PRL release from a prolactinoma is stimulated by Thyrotropin Releasing Hormone (TRH) and inhibited by Dopamine (DA). Stimulation by TRH is dose-dependent with maximal effect at 10^-7 M TRH. Inhibition by DA is also dose-dependent, as