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Possible autocrine enkephalin regulation of catecholamine release in human pheochromocytoma cells

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ABSTRACT

Aims: Pheochromocytomas are catecholamine-secreting tumors that also synthesize and secrete several neuropeptides, including opioids. A negative regulation of catecholamine secretion by opioids has been postulated in chromaffin cells. However, results obtained so far are contradictory when referred to human pheochromocytomas. The aim of this study was to define the role of locally produced enkephalins on catecholamine release in human pheochromocytoma cells.
Main methods: Cells obtained from eleven human pheochromocytomas of different genetic origins were cultured for 5 days. Cultures were maintained under basal condition or under enkephalin, dexamethasone and naloxone alone or in combination with enkephalin or dexamethasone-stimulated conditions. Catecholamine and enkephalin levels in the culture medium were measured by HPLC-ED and RIA respectively.
Key findings: Enkephalin induced a decrease in norepinephrine levels in all tumor cultures. Dexamethasone treatment, which increased enkephalin levels, also decreased catecholamine levels. On the other hand, the addition of naloxone to the cultures reverted to normal the inhibitory action exerted by enkephalin and dexamethasone treatments.
Significance: These results suggest the existence of an autocrine negative regulatory loop exerted by enkephalin on norepinephrine release in human pheochromocytoma cells.

Introduction

Pheochromocytomas are catecholamine-secreting tumors derived from chromaffin cells most commonly found in the adrenal medulla and that usually present with hypertension. Although the majority (90%) of tumors are sporadic, they may also occur in association with other syndromes like MEN2A/2B, neurofibromatosis, von Hippel–Lindau syndrome (Bausch et al., 2006) and succinate dehydrogenase complex II deficiency (Gimm et al., 2000; Astuti et al., 2001a,b).

Pheochromocytomas secrete norepinephrine, epinephrine and, to a lesser extent, dopamine. The investigation of catecholamine secretion on human pheochromocytomas has been mainly conducted in vivo. Few in vitro studies are available and the existing ones mainly describe catecholamine content of human pheochromocytoma cells after a long period in culture. In those studies, a loss or alteration of storage granules (Misugi et al., 1968; Jarry et al., 1989) and a decrease in catecholamine content (Tischler et al., 1984; Jaques and Tobes, 1986) have been observed with the passing of time. Evidence about hormonal regulation of catecholamine production by human pheochromocytoma cells in culture is scarce and it has also been obtained in long-term cultures. In this context, it has been shown that nerve growth factor decreases catecholamine content and induces neurite outgrowth in 21-day-old cultures (Tischler et al., 1984). Furthermore, it has been shown that a variety of structurally related steroids, which include dexamethasone, induce an elevation of catecholamine content in 25-day-old cultures (Brown et al., 1998). To our knowledge, no reports are available on the hormonal regulation of human pheochromocytoma cells in culture during the initial culture period.

In addition to catecholamines, adrenal chromaffin cells synthesize and secrete several neuropeptides, including opioids. It has been shown that opioids are co-localized and co-released with catecholamines in bovine adrenal cells (Viveros et al., 1980; Livett et al., 1981). Many actions of opioids on catecholamine secretion have been reported. In bovine adrenal chromaffin cells it has been shown that some opioids inhibit nicotine-stimulated catecholamine release (Kumakura et al., 1980) while dynorphin and Leu5-enkephalin inhibit endogenous catecholamine secretion (Marley et al., 1986). Also, a modulation of Ca^{2+} channels by Met-enkephalin and ATP has been shown in human adrenal chromaffin cells (Gandía et al., 1998) and an inhibitory effect of Met-enkephalin on acetylcholine-stimulated catecholamine secretion has been shown in the rat adrenal gland (Jarry et al., 1989). As for tumor chromaffin cells, it has been shown in PC12 cells – a rat pheochromocytoma cell line – that κ opioid agonists suppress basal cell proliferation and dopamine secretion (Venihaki et al., 1996a,b), catecholamine biosynthesis (Takekoshi et al., 2000) and basal and nicotine-induced catecholamine secretion (Dermitzaki et al., 2001).

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All these effects may be mediated via opioid receptors present at the plasma membrane of chromaffin cells. The presence of μ, δ and κ opioid receptors in bovine adrenal medulla and in human pheochromocytoma cell membranes has been demonstrated (Castanas et al., 1983, 1984; Kampa et al., 1999). Considering that the role of opioids on catecholamine secretion in human pheochromocytoma has not been clearly demonstrated, we decided to analyze the existence of a possible regulation of catecholamine release by enkephalin in human pheochromocytoma cells in short-term cultures.

Materials and methods

Surgical specimens

Tumor tissue was obtained under aseptic conditions from eleven pheochromocytoma patients. The six male and the five female patients ranged in age from 8 to 61 years at the time of surgery. The diagnosis of pheochromocytoma was made on the basis of accurate laboratory determinations of urinary and/or plasma catecholamines and their metabolites and confirmed by histology. Five were sporadic tumors (#1–#5), three were MEN2A (#6–#8), one was von Hippel–Lindau syndrome (#9), one was succinate dehydrogenase complex II subunit D (SDHD) (#10) and one was succinate dehydrogenase complex II subunit B (SDHB) (#11).

Cell isolation

Tumor tissue was carefully trimmed free from adjacent adrenal cortex and surrounding tissue under sterile conditions. Tissue was minced into fragments measuring approximately 2 mm^3. The minced tissue was placed in a sterile beaker and digested with 0.1% collagenase and 180 μg/ml deoxiribonuclease in Dulbecco's modified Eagle's medium and nutrient mixture Ham's F12 (1:1) supplemented with 20 mM HEPES, 1.8 mg/ml sodium bicarbonate, 100 IU/ml penicillin and 2.5 μg/ml amphotericin B. The enzymatic digestion was performed at 37 °C under an atmosphere of 5% CO_2 and 95% air for 10 min with constant stirring. At the end of the incubation period three volumes of fresh medium were added, the supernatant was collected by aspiration, transferred to a sterile tube and cells were collected by centrifugation at 200×g for 10 min at 4 °C. Cells were resuspended in 1 ml of fresh medium. Crude Cell Fraction was saved. Remaining tissue was submitted to two collagenase treatments under the same experimental condition described above and two additional Crude Cell Fractions were obtained. Crude Cell Fractions were gathered and seeded on top of a 60% Percoll solution and centrifuged at 800 ×g for 30 min at 4 °C. This procedure resulted in the removal of red blood cells. Cells obtained in the 0%–60% interface were collected, washed with fresh medium and centrifuged at 600 ×g for 5 min at 4 °C. Pheochromocytoma cells were resuspended in fresh medium supplemented with 5% fetal bovine serum and 10% horse donor serum.

Culture conditions

Cells (5×10^5 cell/cm^2) were seeded on 24-multiwell tissue culture plates (Falcon Primaria, BD, Franklin Lakes, NJ, USA). Cultures were performed at 37 °C under an atmosphere of 5% CO_2 and 95% air for 5 days. After a 24-hour culture period (Day 1), medium was partially changed every 48 h with or without the addition of Met-enkephalin 10 nM; dexamethasone 10 μM or naloxone 10 μM during the entire

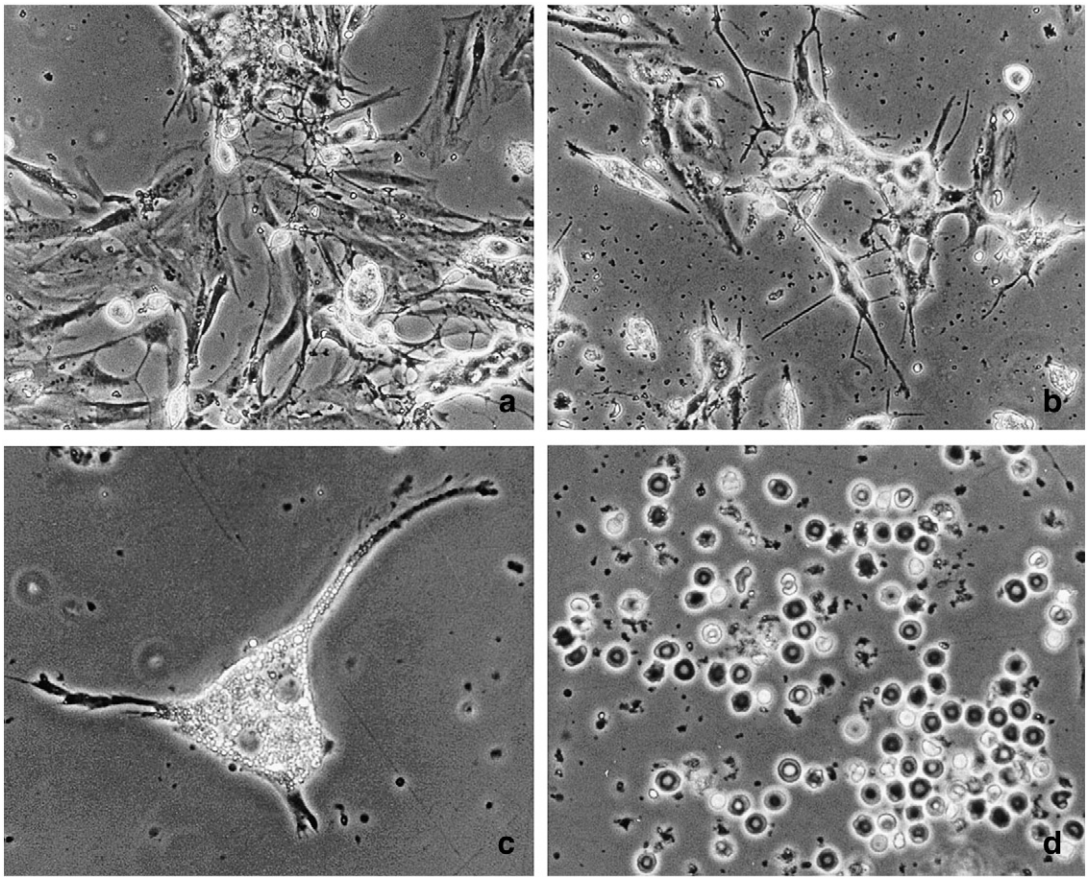

Fig. 1. Morphological characteristics of human pheochromocytoma cells in culture. a) Cells attached to the surface of the plate, growing in clusters and showing epithelial-like characteristics; b) cells attached to the plastic surface showing cytoplasmic processes; c) cells attached to the plastic surface showing numerous cytoplasmic refringent granules; d) cells growing in suspension with round, phase-dark characteristics.