

The amphicrine pancreatic cell line, AR42J, secretes GABA and amylase by separate regulated pathways

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Treatment of AR42J cells with dexamethasone leads to an enhanced formation of amylase-containing granules and facilitates their regulated secretion. Besides the exocrine properties, AR42J cells possess a specific uptake system for [3 H]GABA. The stored GABA can be released upon potassium depolarisation in a Ca^{2+} -dependent manner. After treatment with dexamethasone, potassium depolarisation fails to release GABA, but instead causes a Ca^{2+} -dependent secretion of amylase. Since vesicles similar to small synaptic vesicles of neurons have been identified in AR42J cells, we suggest that the regulated GABA release is mediated by this vesicle type. It is tentatively speculated that other epithelial cells, which also contain small synaptic vesicles and amino acid neurotransmitters, may release them in a similar fashion.

GABA; Small synaptic vesicle; Amylase; Regulated secretion; AR42J cell

1. INTRODUCTION

The pancreatic cell line, AR42J, derived from a rat pancreatic acinar carcinoma, is widely used for studies of exocrine secretion. The cells store amylase in granules, which can be released after stimulation with bombesin, cholecystokinin (CCK), insulin and gastrin. However, regulated secretion is only observed after a longterm treatment with dexamethasone [1–4].

AR42J cells express, in addition to the above mentioned granules, a vesicle type, which resembles the small synaptic vesicles (SSV) of neurons, as revealed by immunoreplica analysis and immunoelectron microscopy [5]. In addition, AR42J cells contain considerable amounts of amino acid neurotransmitters (γ -aminobutyric acid (GABA), glycine and glutamate) [5]. These data suggest the existence of a vesicle type similar to SSV of neurons in AR42J cells. So far, studies concerning the function of these vesicles are lacking. Here we demonstrate that AR42J cells secrete GABA and amylase by two separate pathways.

2. MATERIALS AND METHODS

2.1. Materials

Bombesin, cholecystokinin octapeptide (CCK8), carbachol, dexamethasone, nipecotic acid, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) and aminooxy acetic acid (AOAA) were purchased from Sigma, München, Germany. [3 H]GABA (specific activity 60 Ci/mmol) was from NEN, Dreieich, Germany. The Phadebas amylase assay was obtained from Pharmacia, Freiburg, Germany. The BCA protein determination kit was from Pierce, Karlsruhe, Ger-

many. DMEM, and trypsin/EDTA solution were from Gibco, and foetal calf serum from Biochrom, both Berlin, Germany. All other chemicals were of the purest grade commercially available.

2.2. Methods

AR42J cells were obtained from the American Tissue Type Collection and cultivated as given elsewhere [5]. Treatment with dexamethasone (10^{-7} M final concentration) was usually performed 48 h after plating for 48 h.

Cells were preincubated in Krebs–Ringer–HEPES (KR HEPES) buffer containing 130 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 11 mM glucose, 10 mM HEPES, pH 7.4. The buffer was exchanged for a fresh one containing 1 mM AOAA and 0.5 μ Ci/ml [3 H]GABA and the cells were incubated for 2 h at 37°C. Following three washes for 5 min with fresh KR HEPES buffer, the cells were removed from the culture dish by a short trypsin treatment and distributed to incubation vials. After 20 min at 30°C the medium was removed by a short centrifugation step, followed by aspiration. The cells were resuspended in fresh KR HEPES containing the various secretagogues or in buffer where sodium was replaced by potassium. The stimulation was stopped after 20 min at 30°C by centrifugation. Radioactivity was estimated in the supernatant and in the cell pellets after lysis with lysis buffer containing 130 mM Tris-HCl, 10 mM $CaCl_2$, 75 mM NaCl, pH 8, supplemented with 0.2% Triton X-100. [3 H]GABA release was given as a percentage of the radioactivity present at the beginning of the stimulation. The amount of amylase released and retained by the cells was determined in an aliquot of the supernatant or the cell lysate using the Phadebas assay. Amylase release was given as a percentage of total amylase present at the start of the experiment. Protein was determined using the BCA protein determination kit. Values usually represent the means of three samples \pm S.D.

3. RESULTS

AR42J cells took up [3 H]GABA applied to the medium. Between 2–4 pmol GABA/mg protein was associated with the cells. About 90% of the uptake was sodium dependent. Addition of 1 mM nipecotic acid or 20 μ M FCCP caused a 40% or 95% inhibition of the GABA

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uptake, respectively. Pretreatment of cells with dexamethasone (100 nM for 48 h) enhanced the sodium-dependent GABA uptake 1.5-fold, but the inhibition by nipecotic acid was reduced to about 20% or less in comparison to untreated cells. Elevating the K^+ concentration in the extracellular medium released [3H]GABA from AR42J cells. A 1.5–2-fold stimulation over basal release was observed (Fig. 1, see also Tables I and II). Addition of $CdCl_2$ (50 μM), which blocks voltage-dependent Ca^{2+} channels, did not affect basal GABA release ($10.5 \pm 0.4\%$ in the absence and $8.5 \pm 1.4\%$ in the presence of $CdCl_2$) but abolished the GABA secretion stimulated by 50 mM potassium ($15.7 \pm 2\%$ in the absence and $7 \pm 0.8\%$ in the presence of $CdCl_2$).

Comparing untreated and dexamethasone-treated AR42J cells, it turned out that an elevated K^+ concentration only released GABA from control cells. No stimulated GABA secretion was observed after treatment with dexamethasone. Instead, dexamethasone-treated cells showed an increased amylase secretion, which was not observed in untreated cells (Table I, Fig. 1 and 2). The K^+ -stimulated release of either GABA from untreated or amylase from dexamethasone AR42J cells depended on the extracellular Ca^{2+} -concentration (Table II).

Secretagogues, like bombesin and CCK8, failed to stimulate GABA secretion from AR42J cells. As expected these secretagogues stimulated amylase from dexamethasone-treated cells (data not shown). Similarly, carbachol released amylase only in dexamethasone-treated AR42J cells, but did not affect GABA secretion. Nicotine had no effect on the release of either secretory product (Fig. 3). The carbachol-induced amylase secretion was due to muscarinic receptor activation, for it could be blocked by atropine. Using dexamethasone-treated cells, 20 μM carbachol stimulated amylase secretion from $1.5 \pm 0.3\%$ to $3.3 \pm 0.3\%$. In the presence of atropine, amylase secretion was $1.5 \pm 0.2\%$ and in combination with carbachol $1.4 \pm 0.1\%$.

Table I

Comparison of dexamethasone-treated and untreated AR42J cells with respect to K^+ -stimulated secretion of [3H]GABA or amylase

K^+ (mM)	[3H]GABA		Amylase	
	–	dex	–	dex
25	110 \pm 9.3	100 \pm 12	104 \pm 3.7	182 \pm 32
50	162 \pm 21	88 \pm 32	122 \pm 11	238 \pm 15
100	156 \pm 8	88 \pm 3	118 \pm 17	365 \pm 12

AR42J cells, treated with dexamethasone (100 nM) for 48 h, or control cells were labeled with [3H]GABA and further handled as given in Materials and Methods. For better comparison, values are expressed as a percentage of the control. Release from cells treated with 5.9 mM K^+ containing normal KR HEPES was set at 100% and corresponded to the data given in Figs. 1–3.

4. DISCUSSION

Besides neurons, various neuroendocrine cells have been reported to express the GABA-synthesizing enzyme, glutamate decarboxylase, and to contain GABA ([6–12], for a review see [13]). Furthermore, many non-neuronal cells take up GABA by a sodium-dependent, carrier-mediated process, amongst them glial [14], pancreatic [15] and adrenal chromaffin cells [8,16]. In the central nervous system, GABA is stored in SSV and released by exocytosis from GABAergic neurons acting as an inhibitory transmitter via specific receptors (for a review see [17]). However, the functional properties of the GABA pools in non-neuronal cells are poorly understood. In bovine adrenal chromaffin cells, a stimulated GABA release was reported [8], which could not be reproduced by another group [16].

Evidence that non-neuronal cells possess a vesicle type comparable to the SVV of neurons, which could store amino acid neurotransmitters, came from very recent studies with AR42J cells. The amphicrine properties of AR42J cells, characterized electronmicroscopically by dense core granules and synaptophysin-containing small vesicles with an electron translucent content, suggest the presence of two secretory pathways in these cells [5]. In the same line, various neoplastic neuroendocrine cells also contain large amounts of amino acid neurotransmitters and express integral membrane proteins of SSV, suggesting, again, the existence of a vesicle type analogous to neurons [18,19].

Here we show for the first time, in a permanent cell line, that AR42J cells contain not only the ingredients for vesicular storage and release [5], but do release GABA by regulated secretion. A stimulated release was only observed by potassium depolarisation whereas other secretagogues failed to release GABA. More sur-

Table II

Ca^{2+} dependency of K^+ -stimulated release of [3H]GABA from untreated^a or of amylase from dexamethasone-treated^b AR42J cells

K^+ (mM)	[3H]GABA release (% of control) ^a		Amylase release (% of control) ^b	
	with Ca^{2+}	without Ca^{2+}	with Ca^{2+}	without Ca^{2+}
23	124 \pm 2.3	–	–	–
40	140 \pm 5.6	–	–	–
75	166 \pm 7.8	80 \pm 4.7	–	–
100	207 \pm 2.7	86 \pm 1.4	211 \pm 20	89 \pm 10

^a Preloaded AR42J cells were stimulated with KR HEPES containing the various K^+ concentrations given, either in the presence of 2.5 mM Ca^{2+} or in its absence. Values are expressed as a percentage of the control (see Table I). The basal release in the absence of Ca^{2+} increased 2-fold for so far unknown reasons.

^b AR42J cells were treated with dexamethasone (100 nM) for 48 h and amylase release was stimulated either in the presence or absence of Ca^{2+} . Basal amylase secretion was not affected by the omission of Ca^{2+} .

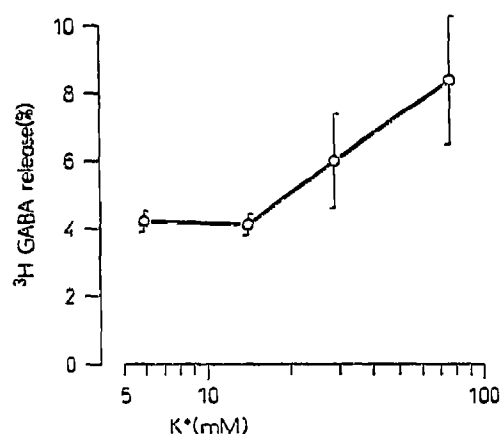


Fig. 1. K⁺-stimulated [³H]GABA release by AR42J cells. Preloaded cells (see Materials and Methods) were stimulated with the K⁺ concentrations given on the abscissa.

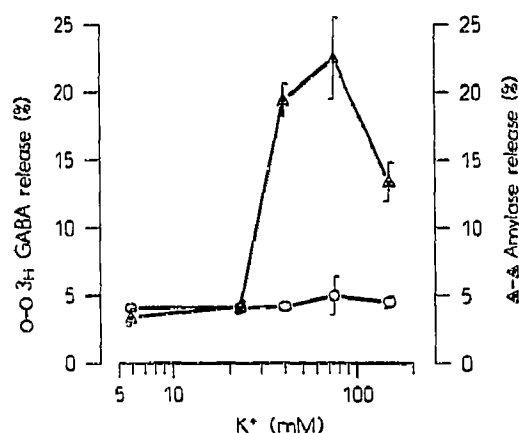


Fig. 2. K⁺-stimulated release of amylase from dexamethasone-treated AR42J cells. AR42J cells were treated with dexamethasone (100 nM) for 48 h. Cells were labelled with [³H]GABA and stimulated with various K⁺-concentrations given on the abscissa. [³H]GABA and amylase were estimated from the same sample.

prisingly, only untreated cells showed a regulated GABA release, which disappeared after treatment with dexamethasone. Dexamethasone stops proliferation and leads to an exocrine phenotype, indicated by an increase of zymogen-like granules and amylase secretion ([1,2,21], and also this paper). However, neither the uptake of GABA nor the synaptophysin content (the most prominent marker of SSV) is affected after treatment with dexamethasone [5], and may therefore not explain our findings. Furthermore, the lack of a stimulated GABA secretion in dexamethasone-treated cells is probably not due to a disappearance of voltage-dependent Ca²⁺ channels [20], since a stimulated amylase secretion was still observed. Probably, other vesicle-

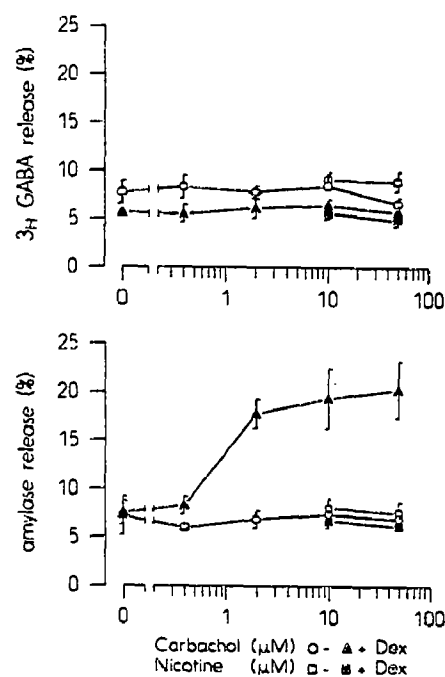


Fig. 3. Effects of carbachol and nicotine on [³H]GABA and amylase secretion from untreated or dexamethasone-treated AR42J cells. AR42J cells treated with dexamethasone (100 nM) control cells were labelled with [³H]GABA. The cells were stimulated for 20 min at 30°C with the carbachol or nicotine concentrations given at the abscissa. Radioactivity and amylase in the supernatant and in the cell lysate were estimated from the same sample.

plasma membrane component are affected by dexamethasone.

In conclusion, AR42J cells represent a model system for the study of secretion for two reasons: (i) two separate regulated secretory processes can be studied simultaneously within the same cell; (ii) the differential effects on secretion induced by dexamethasone may be used to learn more about the regulation of these two separately regulated secretory pathways.

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