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Research paper

Proteomic characterization of Jurkat T leukemic cells after dopamine stimulation: A model of circulating dopamine-sensitive cells

Tiziana Alberio [a], Claudia Anchieri [a], Luca Piacentini [b], Giovanna Gentile [c], Maurizio Simmaco [d], Mara Biasin [b], Mauro Fasano [a,*]

[a] Department of Structural and Functional Biology, and Centre of Neuroscience, University of Insubria, via Alberto da Giussano 12, 21052 Busto Arsizio, Italy
[b] Chair of Immunology, DISC LITA Vialba, University of Milan, Via G.B. Grassi 74, 20157 Milano, Italy
[c] Department of Biochemical Sciences, Advanced Molecular Diagnostic Unit, 2nd School of Medicine, Sapienza University of Rome, Sant'Andrea Hospital, Via di Grottarossa 1035, 00189 Roma, Italy
[d] NESMOS Department, Advanced Molecular Diagnostic Unit, 2nd School of Medicine, Sapienza University of Rome, Sant'Andrea Hospital, Via di Grottarossa 1035, 00189 Roma, Italy

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ABSTRACT

T-cells are circulating dopamine-sensitive cells and may mirror, at the peripheral level, biochemical modifications occurring in dopaminergic neurons. The human CD4+ T leukemic Jurkat cell line has been thoroughly used and characterized as a suitable cell model to investigate T-cell signaling and apoptosis. Here, we describe their characterization as a model of circulating dopamine-sensitive cells and their response to a dopamine challenge by analyzing changes in the cell proteome. Jurkat cells express both D1- and D2-like dopamine receptors and both membrane and vesicular transporters, while they lack D4 receptor and tyrosine hydroxylase expression. A saturating, non-toxic, non-oxidant 50 μM dopamine challenge induces the upregulation of an interacting chaperone network known to protect specifically dopaminergic neurons, thus validating T-cells as a biomarker discovery source in Parkinson's disease. Remodeling of the distribution pattern of β-actin and 14-3-3 isoforms is consistently observed upon dopamine treatment. As a whole, dopamine-specific alterations here observed might represent a biosensor for dopamine response at the peripheral level.

1. Introduction

The functions of immune cells are regulated not only by cytokines, but also by several neurotransmitters. In particular, peripheral blood lymphocytes possess a complex dopaminergic regulatory system [1–3]. Human T-lymphocytes express all five dopamine receptors (D1–D5), each of which exerts different actions on the regulation of T-cell functions [2]. In particular, the stimulation of D1/D5 and the consequent increase of cAMP determines the inhibition of cytotoxic CD8+ cells, impairs function and differentiation of T_{reg} cells and induces polarization of naive CD4+ cells toward T helper 17 [4–7]. On the other hand, stimulation of D3 receptors controls T-cell adhesion and migration, induces differentiation of CD8+ cells into cytotoxic T-lymphocytes and polarization of naive CD4+ cells toward T helper 1. Finally, D2 and D4 receptors are effective in regulating the activation and differentiation of naive CD4+ cells, *e.g.,* by promoting the polarization toward T_{reg} cells (D2) or by triggering T-cell quiescence (D4) [2,8–10].

T_{reg} and effector T-cells express vesicular monoamine transporters VMAT1 and VMAT2, thereby they can store dopamine and release it through a pseudo-synaptic mechanism. However, only T_{reg} cells constitutively express tyrosine hydroxylase (TH) and are therefore able to synthesize dopamine [4]. Interestingly, effector T-cells are able to cross the blood–brain barrier and to enter the central nervous system (CNS) where they are exposed to a variety of neurotransmitters [2,11]. On this basis it has been proposed that T-lymphocytes may constitute an amenable source of peripheral biomarkers for neurodegenerative disorders, in particular for

Abbreviations: 2-DE, two-dimensional electrophoresis; CHAPS, 3-[(3-cholamido)dimethylammonio]-1-propanesulfonic acid; CNS, central nervous system; DAT, dopamine transporter; DTT, dithiothreitol; FBS, fetal bovine serum; GO, gene ontology; IEF, isoelectrofocusing; IPG, immobilized pH gradient; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate buffered saline; PD, Parkinson's disease; PVDF, polyvinylidene difluoride; SDS, sodium dodecylsulfate; TBS, Tris buffered saline; TBS-T, Tris buffered saline–Tween 20; TFA, trifluoroacetic acid; TH, tyrosine hydroxylase; TOF, time-of-flight; VMAT, vesicular monoamine transporter.

* Corresponding author. Tel.: +39 0331 339450; fax: +39 0331 339459.
E-mail addresses: tiziana.alberio@uninsubria.it (T. Alberio), claudia.anchieri@gmail.com (C. Anchieri), luca.piacentini@unimi.it (L. Piacentini), giovanna.gentile@uniroma1.it (G. Gentile), maurizio.simmaco@uniroma1.it (M. Simmaco), mara.biasin@unimi.it (M. Biasin), mauro.fasano@uninsubria.it (M. Fasano).

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Parkinson's disease (PD), where the dopaminergic system is impaired [12].

Immune mechanisms may contribute to neuronal damage, as suggested by the immune disturbances reported in PD [13], as well as by the demonstration of chronic neuroinflammatory processes in the brain of PD patients [14]. Moreover, peripheral blood cells have been shown to share some of the changes exhibited by nigral neurons [15–18]. Mutations in the *parkin* gene (*PARK2*) render lymphocytes more susceptible to dopamine- and iron-mediated apoptosis [19]. α-Synuclein, a major PD-related protein, is overexpressed in lymphocytes of sporadic PD patients and its expression correlates with apoptotic markers [20]. Oxidative-stress-induced apoptosis has been reported in lymphocytes of two patients carrying the A53T α-synuclein mutation [21]. The expression of transcription factor Nurr-1, which is critical in the development and maintenance of the dopaminergic system in the CNS, is reduced both in PD midbrains and in lymphocytes of parkinsonian patients [22]. A marked alteration in calcium homeostasis has been reported in lymphocytes of PD patients affected by levodopa-induced dyskinesias with respect to patients under levodopa treatment that do not show dyskinetic movements [15]. It is thus realistic that biochemical alterations at the SNpc level caused by genetic defects may also have a counterpart at the T-cell level [12]. In other words, mutations that lead to specific damage of dopaminergic neurons may produce functional alterations in dopamine-sensitive peripheral cells (i.e., T-cells).

In recent years proteomics was used as a hypothesis generating tool in neuroscience to investigate pathogenetic mechanisms and to discover new biomarkers of neurodegenerative disorders. Nevertheless, no protein markers have been proposed for PD diagnosis and/or PD subtype classification yet [12,23–27]. In the absence of reliable, affordable and readily available laboratory tests, the diagnosis of PD is currently based on the observation of clinical symptoms that become noticeable when more than 80% neurons in the substantia nigra are lost [28]. Disease biomarkers are particularly useful if they are present in body fluids, like blood (plasma and blood cells) or urine [23,26]. T-cells are easy to obtain from patients with little discomfort and may represent molecular sensors to evaluate gene expression modification in physiological and pathological conditions, thereby providing a unique and easily available biological model for integrated studies of gene expression in humans [29–31]. The first proteomics investigation reported so far in lymphocytes of sporadic PD patients has revealed cytoskeletal protein dysregulation and oxidative stress [31]. Since genetic defects that cause a parkinsonian phenotype may actually determine biochemical alterations at the T-cell level, proteomics of T-cells may represent a powerful route to track a genetic origin, be it already known or still unidentified [12,29,31].

The Jurkat human CD4+ T-cell leukemia cell line has been thoroughly used and characterized as a suitable cell model to investigate T-cell signaling and apoptosis [32]. Nevertheless, its characterization as a model of circulating dopamine-sensitive cells has not been reported before. As a cell line, Jurkat cells have the great advantage to be easily expanded, thus generating highly reproducible conditions that cannot be obtained using T-cell cultures. Consequently, Jurkat cells could be used to identify dopamine-sensitive biochemical mechanisms that may be present in T-cells as well. In this regard, we here report the biochemical characterization of the dopaminergic system in Jurkat cells and the proteomic characterization of their response to exposure to a saturating, non-toxic dopamine concentration. Proteins responding to dopamine are expected to change when the dopaminergic system is somehow altered, therefore they may represent suitable biosensors of an impaired dopaminergic system in T-cells from PD patients.

2. Experimental procedures

2.1. Cells

The Jurkat T-cell leukemia cell line was maintained at 37 °C in a 5% CO_2 humidified atmosphere in RPMI 1640, supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. All cell culture media and reagents were from PAA (Austria). Stock flasks were transferred for culture twice weekly or as required to maintain optimal cell growth.

2.2. RNA extraction, retrotranscription and PCR

RNA was extracted using NucleoSpin RNA II (Macherey–Nagel, Germany) following manufacturer's instructions and treated with rDNase in order to remove genomic DNA. Five micrograms of RNA were reverse transcribed into first-strand cDNA in a 20 μl final volume using the Maxima™ First Strand cDNA Synthesis Kit (Fermentas, Canada). PCR was performed by Real-Time PCR (DNA Engine Opticon 2; MJ Research, USA) using SYBR Green PCR mix (5Prime, Germany). The expression of D1 receptor, D2 receptor splicing variants 1 and 2, D3 receptor splicing variant a, D4 receptor, D5 receptor, dopamine transporter (DAT), VMAT1, VMAT2 and TH was evaluated using the primers reported in Table 1. Primer design was performed by using Primer3 software (http://primer3.sourceforge.net/) and manually adjusted if needed. Primer specificity was tested by BLAST database search against nucleotide reference NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and experimentally by the positive control amplification. Optimal PCR conditions were identified for each primer pair. Reactions were incubated at 95 °C for 2 min, then 40 cycles at 95 °C for 15 s, 62 °C for 20 s and 68 °C for 20 s. As positive control, cDNA from the catecholaminergic human neuroblastoma SH-SY5Y cell line was used and amplified under the same conditions. The specificity of the amplified products was checked by the melting temperature curve analysis and the expected size of the fragments was further visualized in a 2% agarose gel stained with ethidium bromide. Results were confirmed by three independent replicates. Furthermore, PCR efficiency was determined using serial dilution of control templates obtained by the amplification of positive controls. The dilutions were used as templates for PCR reactions. All PCR

Table 1
Sequences of specific primers: F = forward primer; R = reverse primer.

Gene	Primer Sequence	Amplicon size (bp)
D1 F	ATGGACGGGACTGGGCTGGT	91
D1 R	GGAGCGTGGACAGGATGAGCA	
D2(1) F	AGCCTCCACTCTCCGCCTG	102
D2(1) R	CCACTAAAGGGCAACTGTACTCACC	
D2(2) F	CGCCGGGCAGCCTCCTTTAG	156
D2(2) R	CCATCGTCTCCTTCTACGTGCCC	
D3(a) F	GGCACTCCCCGAGGTTGCAG	116
D3(a) R	CGGAATTCCCTGAGTCCCACC	
D4 F	GCCGACCTCCTCCTCGCTCT	169
D4 R	CGAACCTGTCCACGCTGATGG	
D5 F	TCTACCGCATCGCCCAGGTG	189
D5 R	GCAGCCAGCAACACACGAAGA	
VMAT1 F	GCTTTCTTCAACAGCCACGGTG	170
VMAT1 R	CTGTGGTGGTGCCAATTGTGC	
VMAT2 F	CCATTGCGGATGTGGCATTT	166
VMAT2 R	TCTTCTTTGGCAGGTGGACTTCG	
DAT1 F	AGCCTGCCTGGGTCCTTTCG	106
DAT1 R	AGTGGCGGAGCGTGAACTGG	
TH F	CGACCCTGACCTGGACTTGGA	142
TH R	GGCAATCTCCTCGGCGGTGT	

reactions displayed similar and optimal efficiency for each primer specific amplification protocol.

2.3. 2-DE electrophoresis and quantitative analysis

Jurkat cells treated or not with 50 μM dopamine in the presence of 140 U/ml catalase [33] for 24 h were collected by centrifugation, lysed with 200 μl lysis solution (7 M urea, 2 M thiourea, 4% w/v CHAPS, 0.5 μl protease inhibitor mix) and centrifuged (13,000 g, 30 min, 10 °C). Proteins were collected in the supernatant and their concentration was determined using the Bio-Rad Protein Assay (Bio-Rad, USA).

2-DE was performed according to Görg [34], with minor modifications. Samples (about 500 μg) were diluted to 250 μl with a buffer containing 7 M urea, 2 M thiourea, 4% CHAPS, 0.5% IPG buffer 3–10 NL, 2 mM tributylphosphine and traces of bromophenol blue, and loaded on 13 cm IPG DryStrips with a non-linear 3–10 pH gradient by in-gel rehydration (1 h at 0 V, 10 h at 50 V). Isoelectrofocusing (IEF) was performed at 20 °C on IPGphor (GE Healthcare, UK) according to the following schedule: 2 h at 200 V, 2 h linear gradient to 2000 V, 2 h at 2000 V, 1 h of linear gradient to 5000 V, 2 h at 5000 V, 2 h linear gradient to 8000 V and 2 h and 30 min at 8000 V. Immobilized pH gradient (IPG) strips were then equilibrated for 2 × 30 min in 50 mM Tris–HCl pH 8.8, 6 M urea, 30% glycerol, 2% SDS and traces of bromophenol blue containing 1% DTT for the first equilibration step and 2.5% iodoacetamide for the second one. SDS-PAGE was performed using 11% 1.5 mm thick separating polyacrylamide gels without stacking gel, using SE 600 system (Hoefer, USA). The second dimension was carried out at 45 mA/gel at 18 °C. Molecular weight marker proteins (11–170 kDa from Fermentas, Canada) were used for calibration.

Gels were stained with Coomassie Brilliant Blue R-350, scanned with an Epson Perfection V750 Pro transmission scanner (Epson, Japan) and analyzed with ImageMaster 2D Platinum V5.0 software package (GE Healthcare). Spots were automatically detected by the software and manually refined afterward; gels were matched and the resulting clusters of spots confirmed manually. Spots were quantified on the basis of their relative volume (spot volume normalized to the sum of the volumes of all the representative spots in the same gel) and those that consistently and significantly varied between controls and dopamine-treated samples were identified by Student's *t*-test analysis with a threshold $p \leq 0.05$.

2.4. Protein identification by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry

Protein spots were excised manually from the gel, washed with high-purity water and destained twice with 100 μl of 50 mM NH_4HCO_3/50% acetonitrile for 10 min. Spots were dehydrated with 100% acetonitrile and swollen at room temperature in 20 μl of 40 mM NH_4HCO_3/10% acetonitrile containing 25 ng/μl trypsin (Trypsin Gold, mass spectrometry grade; Promega, USA). After 1 h, 50 μl of 40 mM NH_4HCO_3/10% acetonitrile were added and digestion proceeded overnight at 37 °C. The generated peptides were extracted with 50% acetonitrile/5% trifluoroacetic acid (TFA; two steps, 20 min each at room temperature), dried by vacuum centrifugation, suspended in 0.1% TFA, passed through micro ZipTip C18 pipette tips (Millipore, USA) and eluted directly with the MS matrix solution (10 mg/ml α-cyano-4-hydroxycinnamic acid in 50% acetonitrile/1% TFA). Mass spectra of the tryptic peptides were obtained using a Voyager-DE MALDI-TOF mass spectrometer (Applied Biosystems, USA). Peptide mass fingerprinting database searching was performed using the mascot search engine (http://www.matrixscience.com) in the NCBInr/Swiss-Prot databases. Parameters were set to allow one missed cleavage per peptide, a mass tolerance of 0.5 Da and considering carbamido-methylation of cysteines as a fixed modification and oxidation of methionines as a variable modification.

2.5. Bioinformatics enrichment and network clustering

The list of identified proteins was fed to PPI spider (http://mips.helmholtz-muenchen.de/proj/ppispider/) in order to determine a statistically-significant interaction network as well as statistically-significant functional association to Gene Ontology (GO) classifications [35].

2.6. Two-dimensional western blotting

2-DE was performed as described in Section 2.3. Gels were transferred to polyvinylidene difluoride (PVDF) membranes at 25 V for 2 h. Membranes were saturated in 5% non-fat milk in TBS–T (0.5 M Tris pH 7.4, 2 M NaCl and 0.1% Tween 20) and incubated with rabbit anti-14-3-3 polyclonal antibody (Zymed, USA), 1:1000 dilution in 5% milk-TBS–T for 1.5 h at room temperature or with mouse anti-β-actin monoclonal antibody (GeneTex, USA). Membranes were then washed with TBS–T and incubated with peroxidase-conjugated anti-rabbit-IgG antibody (Millipore, USA), 1:1000 in 5% milk-TBS–T, or anti-mouse-IgG antibody (GeneTex, USA), 1:3000 in 5% milk-TBS–T, respectively, for chemiluminescence detection (Pierce, USA). Photographic films were scanned with an Epson Perfection V750 Pro transmission scanner (Epson, Japan) and images were analyzed using ImageJ software (http://rsb.info.nih.gov/ij/).

3. Results

To investigate the nature of the dopaminergic system in Jurkat cells, we evaluated the mRNA expression of D1, D2(1) and D2(2) splicing variants, D3(a) splicing variant, D4 and D5 receptors, DAT, VMAT1 and VMAT2 transporters and TH (Fig. 1, upper panel). We selectively evaluated the D3(a) level because the D3(e) splicing isoform does not bind dopamine [36]. Detectable transcript levels of D1, D2(1), D3(a) and D5 receptors, as well as of DAT, VMAT1 and VMAT2 transporters, allowed us to establish the occurrence of a complete dopaminergic system in Jurkat cells. The appropriateness of primers and RT-PCR conditions was verified by checking the expression of the dopaminergic system components in the catecholaminergic human neuroblastoma SH-SY5Y cell line (Fig. 1, lower panel).

Dopamine challenge of Jurkat cells induced quantitative changes in the protein expression pattern, as deduced by image analysis of

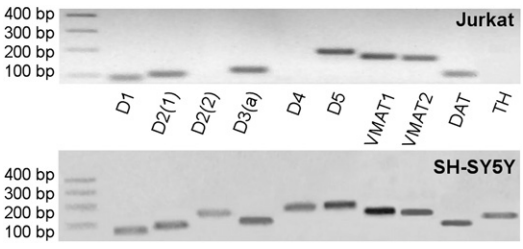

Fig. 1. Expression at the transcriptional level of the dopaminergic system components in Jurkat T leukemic cells and neuroblastoma SH-SY5Y cells (as positive control). Receptors D1, D2(1) and D2(2) splicing variants, D3(a) splicing variant, D4 and D5, transporters VMAT1, VMAT2 and DAT, and tyrosine hydroxylase (TH) in Jurkat cells (upper panel) and SH-SH5Y cells (lower panel) are visualized on a 2% agarose gel stained with ethidium bromide after RNA extraction, reverse transcription and PCR amplification. Results were confirmed by three independent replicates. For further details see Section 2.2 and Table 1.

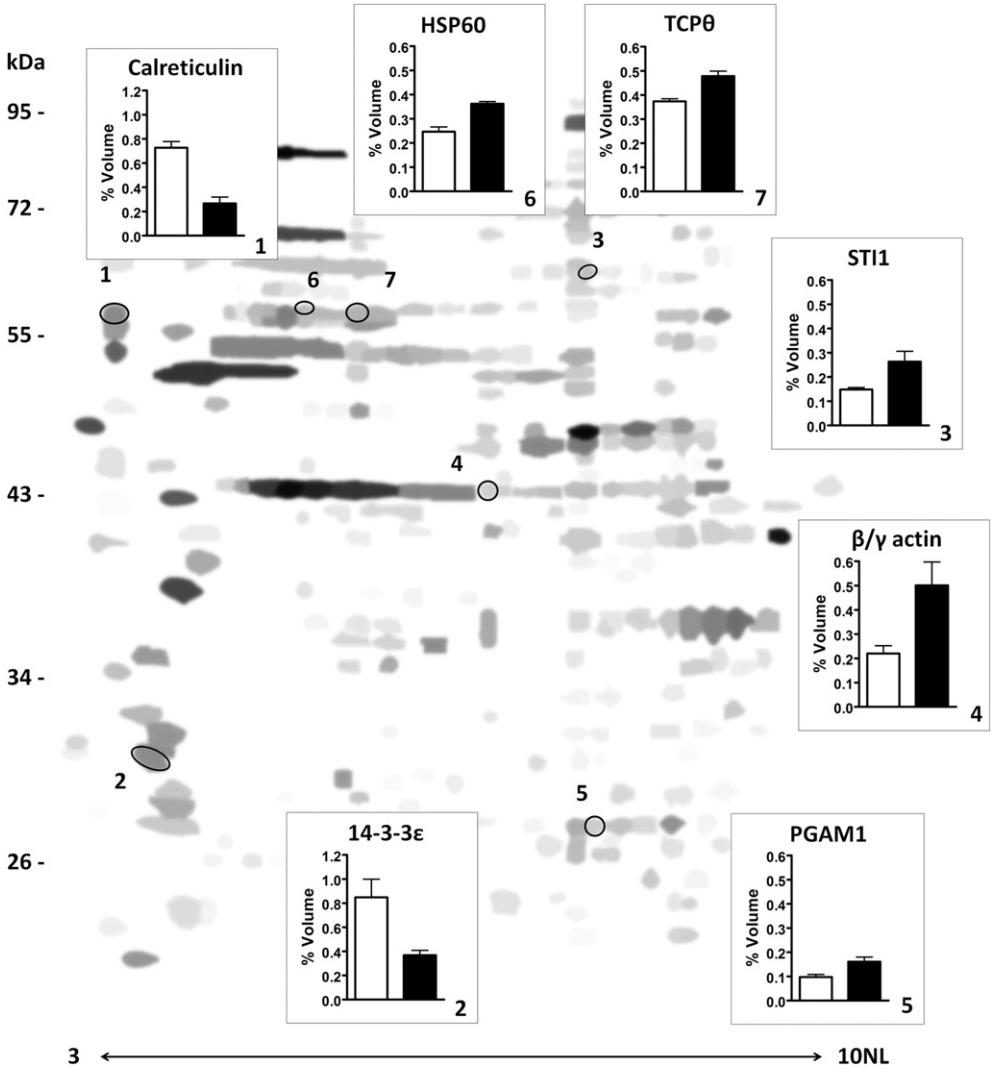

Fig. 2. Representative spots in a synthetic two-dimensional gel of proteins extracted from whole Jurkat cells. The synthetic gel is obtained from Coomassie stained gels (three replicates per experimental condition) by averaging the normalized volumes of spots present in all considered gels. Greyscale refers to mean spot volumes. Spots which display a significant change in intensity after dopamine challenge (50 μM, 24 h) are numbered. Insets report the mean percent spot volumes ± SE in three different gels. Open bars: control cells; filled bars: dopamine-treated cells. For protein identifications see Table 2. For further details and experimental conditions see Section 2.3.

2-DE gels (Fig. 2). A 50 μM dopamine concentration (24 h) was chosen in order to stimulate all receptors and at the same time to load cells through the DAT and VMAT transporter system. Receptor affinity for dopamine is known to range from 5 nM to 5 μM [36]. Thus, a dopamine concentration higher than the highest value of the receptor dissociation constants needs to be used. On the other hand, the dopamine concentration is limited by the appearance of oxidative-stress conditions associated to dopamine auto-oxidation [37]. Cell viability was not affected by the treatment (data not shown), thus confirming that the chosen concentration was not toxic. We observed reproducible changes in the level of 7 spots (Student's *t* test) that were further analyzed by peptide mass fingerprinting. Consequently, we were able to establish that calreticulin and 14-3-3 ε were downregulated by dopamine treatment, whereas STI1, a basic isoform of β/γ-actin, phosphoglycerate mutase 1 (PGAM1), HSP60 and TCP1θ were upregulated (Table 2).

Table 2
Peptide mass fingerprinting identification of differentially expressed proteins in Jurkat cells challenged with 50 μM dopamine.

Spot No.	Protein	Accession No.[a]	Theoretical MW (kDa)	pI	Fold of induction	P[b]	Sequence coverage (%)	Mowse score
1	Calreticulin	P27797	47.9	4.29	2.72↓	0.02	30	83
2	14-3-3 ε	P62258	29.2	4.63	2.30↓	0.05	34	74
3	STI1	P31948	62.6	6.40	1.78↑	0.05	34	119
4	β/γ-actin	P60709	41.7	5.29	1.92↑	0.03	38	86
5	Phosphoglycerate mutase 1	P18669	28.7	6.75	1.65↑	0.02	46	131
6	HSP60	P10809	58.0	5.24	1.47↑	0.01	24	62
7	TCP1θ	P50990	59.5	5.42	1.63↑	0.01	18	98

[a] Uniprot database.
[b] Student's *t* test.

In order to build a comprehensive model to encompass the experimentally identified proteins, we analyzed the entire list in terms of both interaction network and GO classification enrichment using PPI spider [35]. A significant ($p < 0.05$) enriched network for proteins altered by the dopamine challenge is reported in Fig. 3. A significant functional association to Gene Ontology (GO) class "unfolded protein binding" (GO:0051082) was obtained for this network.

The observation of a significant change in a basic isoform of β/γ-actin appeared quite unusual. Therefore, we proceeded to validate the finding by two-dimensional Western blotting. Actually, dopamine treatment markedly affected the 2-DE pattern of β/γ-actin with a shift toward basic forms (Fig. 4, Panel A).

Two members of the 14-3-3 chaperone family seemed to be affected by dopamine treatment: 2-DE analysis revealed the downregulation of 14-3-3 ε (Fig. 2), whereas the network enrichment procedure assigned a central role to 14-3-3 ζ (Fig. 3). However, the high degree of sequence identity (over 70%) among 14-3-3 proteins does not allow the specific assignment to 14-3-3 ε by peptide mass fingerprinting. On the other hand, all members of the 14-3-3 family bind their targets with similar affinity [38], therefore we cannot exclude that 14-3-3 proteins other than ζ might take part to the same network. Thus, we performed two-dimensional Western blotting using an antibody that recognizes all 14-3-3 isoforms (Fig. 4, Panel B). Dopamine treatment induced a global change in isoform distribution. Images were acquired using two different exposure times, in order to discriminate differences in both low- and high-abundance species. By saturating the main component, we were able to observe the downregulation of minor spots following dopamine treatment (Fig. 4, Panel B, left). By lowering the exposure time, on the other hand, the most abundant isoforms appeared as four resolved spots, whose relative levels were modified by dopamine (Fig. 4, Panel B, right).

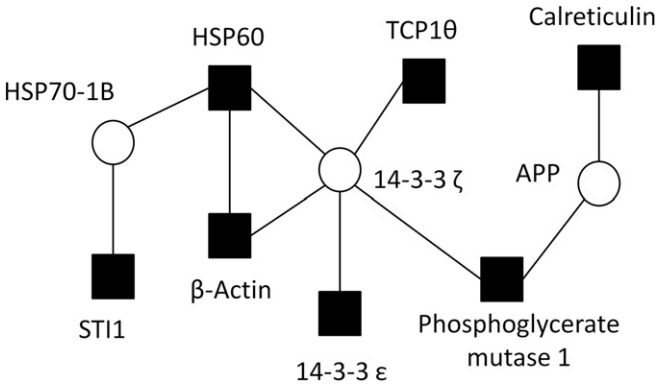

Fig. 3. Protein–protein interaction network enrichment. Filled squares refer to experimentally identified proteins, whereas empty circles indicate enriched proteins (common interactors). The network enrichment procedure was performed using PPIspider (available at http://mips.helmholtz-muenchen.de/proj/ppispider/), $p < 0.05$. For further details see Section 2.5.

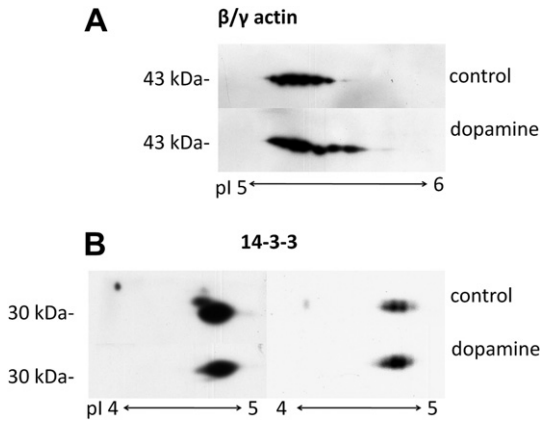

Fig. 4. Two-dimensional Western blot analysis of β-actin and 14-3-3 isoform patterns. Panel A: alteration of the β-actin pattern in control (upper row) and dopamine-treated cells (50 μM, 24 h, lower row). Panel B: alteration of the 14-3-3 pattern in control (upper row) and dopamine-treated cells (lower row) at different exposure times (left: 20 s; right: 5 s). Results were confirmed by three independent replicates. For further details and experimental conditions see Section 2.6.

4. Discussion

Jurkat cells express different components of the dopaminergic system. Both D1- and D2-like receptors are present, thus accounting for a variety of cellular responses following dopamine stimulation. Remarkably, D3(a) and D5 receptors were detected, in addition to what was recently reported [39]. Moreover, they express plasma membrane and vesicular transporters, which makes them capable to internalize dopamine and store it into vesicles. Noteworthy, Jurkat cells are not able to synthesize dopamine as they lack TH expression. The body of these results suggests that Jurkat cells posses a dopaminergic system similar to the vast majority of T-cells [2], TH activity having been reported in T_{reg} cells only [4]. On this basis, Jurkat cells appear to be a suitable model for dopamine-sensitive peripheral cells.

Dopamine stimulation may elicit both receptor- and transporter-mediated responses. For this reason, the dopamine concentration employed here was chosen as the highest possible (without affecting cell viability) in order to induce a wide variety of responses. Indeed, D1 receptors are reported to bind dopamine with a thermodynamic equilibrium dissociation constant in the micromolar range [36]. Although the identification of the transduction mechanisms activated by dopamine could be of interest to understand the role of the neurotransmitter in regulating immune responses [2,39], our purpose was to identify variations at the protein level that altogether constituted a sort of dopamine biosensor. As a matter of fact, dopamine induces a significant change in the Jurkat cell proteome. Specifically, alterations are observed in chaperone proteins, glycolytic enzymes and proteins involved in cytoskeleton maintenance. Upregulation of stress proteins (*e.g.*, HSP60) and of glycolytic enzymes (*e.g.*, phosphoglycerate mutase 1) are commonly identified in proteomics studies and may reflect an unspecific response to a generic stress condition [40], or a proteome adaptation as a response to perturbation in the protein levels caused by stimuli of various origin [41]. β-Actin itself may behave as a proteome balancer in this view, although its deregulation was associated to early stages of pathogenesis in a *Drosophila* model of PD [42]. The actin isoform pattern is not only modified in its principal components, but it shifts toward basic forms. Indeed, the observed upregulation of a basic β/γ-actin form after dopamine treatment is confirmed by two-dimensional Western blotting, in which a general basic shift is observed. Alterations in the 2-DE pattern of β-actin, with the upregulation of a basic isoform, were also described in lymphocytes of PD patients regardless of the therapy [31]. Noticeably, TCP1θ, a subunit of the main actin folding chaperonin CCT [43], is upregulated in Jurkat cells by dopamine treatment.

STI1 and TCP1θ, both upregulated in dopamine-treated cells, may take part to the formation of a large multichaperone complex which recruits further chaperones, such as other TCP1 isoforms and heat shock proteins (*e.g.* HSP70, HSC70, HSP90). This complex has been demonstrated to play an active role in the protection of dopaminergic neurons [44]. Moreover, dopamine treatment of

differentiated PC12 cells induces a marked upregulation of chaperone proteins, suggesting a protective response toward unfolded protein stress [45]. Thus, the upregulation here reported might constitute a specific response of dopamine-sensitive cells. It should be considered that the low dopamine concentration used in this work is not likely to produce the aspecific oxidative-stress conditions that are observed in neuronal dopaminergic cells, when exposed to higher levels [33,37,46].

An independent confirmation of the specificity of the proteome changes observed in dopamine challenged Jurkat cells comes from the bioinformatic network enrichment procedure that clusters in a single network (Fig. 3) all the experimentally identified proteins. Additionally, several proteins among those listed here are interactors of the 14-3-3 protein family, a set of adaptor proteins that regulate many cellular processes, including dopamine synthesis [47]. The PD-related protein α-synuclein is also an interactor of 14-3-3 proteins. Additionally, α-synuclein and 14-3-3 share two regions (29 and 21 residues) with moderate sequence conservation (35% and 29% identity, respectively) and have common interactors such as BAD and protein kinase C [48]. Interestingly, alterations in 14-3-3 proteins have been reported in PD models, including downregulation of 14-3-3 ϵ following overexpression of α-synuclein [49]. Although the assignment to specific 14-3-3 isoforms is merely tentative, the analysis of all the 14-3-3 proteins by two-dimensional Western blotting confirms that a significant, general remodeling of their 2-DE pattern is associated with dopamine treatment. Even if we exclude that the pattern remodeling could be associated with the regulation of dopamine synthesis, given the absence of detectable expression of TH (Fig. 1, upper panel), the observed changes in isoforms distribution could be at the ground of anti-apoptotic pathways activated by dopamine [49]. Due to intrinsic limits of the resolution of 2-DE and of the specificity of peptide mass fingerprinting, a more detailed description of 14-3-3 changes induced by dopamine in Jurkat cells will require further investigation.

5. Conclusions

Jurkat cells react to a non-oxidative, saturating dopamine stimulus by inducing specific changes in a network of chaperone proteins that are able also to interact with 14-3-3 and β-actin. This network is known to protect dopaminergic neurons from the dopamine-associated damage [44].

These findings substantiate the use of T-cells as an appropriate source of peripheral biomarkers in conditions where the dopaminergic system is involved, as it is the case with PD [12]. The evidence of a specific response to dopamine, on the other hand, suggests that Jurkat cells are a useful cell culture model to investigate dopamine-activated biochemical processes. Eventually, the set of proteins whose level is modified by dopamine in Jurkat cells might itself represent a reporter of dopamine response at the peripheral level, for instance in T-cells of PD patients whether or not stimulated with dopamine.

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