ABSTRACT

The aim of this Ph.D. thesis was to analyze the morphine-induced changes of frontal brain cortex protein composition in rats exposed to increasing doses of morphine (10-50 mg/kg) for prolonged period of time (10 days).

The first part of this work was oriented to the analysis of the phenomenon of hypersensitization/superactivation of adenylyl cyclase (AC), which is regarded as one of the crucial molecular mechanisms causing drastic pathological consequences of drug addiction. The increase of AC activity represents a “compensatory” response and is functionally related to the desensitization of G protein response to prolonged morphine exposure of target cells.

The clear desensitization of μ-OR- and δ-OR-stimulated G protein response by morphine was demonstrated in our laboratory by analysis of the dose-response curves of DAMGO and DADLE-stimulated, high-affinity \[^{35}\text{S}]\ GTP\gamma S\ binding in plasma membranes isolated from frontal brain cortex of rats exposed to morphine according to the same protocol as that used in my Ph.D. thesis (10-50 mg/kg, 10 days). The κ-OR-stimulated \[^{35}\text{S}]\ GTP\gamma S\ binding was unchanged. It has been determined the amount of all AC isoforms (AC I-IX) in plasma membranes (PM) isolated from control and morphine-treated rats which were sacrificed 24 hours since the last dose of morphine. This has been performed by the immunoblot analysis using the specific antibodies oriented against AC I-IX. The results indicated the specific up-regulation of AC I (8x) and AC II (2.5x) in plasma membrane fraction isolated by centrifugation in Percoll\textsuperscript{®} density gradient; the amount of AC III-IX was unchanged. The parallel analysis of the same PM samples indicated unchanged level of all the major classes of trimeric G proteins (G\textsubscript{αi1}/G\textsubscript{αi2}, G\textsubscript{αi3}, G\textsubscript{αo}, G\textsubscript{αq}/G\textsubscript{α11}, G\textsubscript{αs}, G\textsubscript{β}) and of the prototypical plasma membrane marker Na, K-ATPase. Importantly and surprisingly, the specific increase of AC I and AC II was not detected in PM isolated from rats exposed to morphine for 10 days but sacrificed 20 days since the last dose. Thus, the morphine-induced increase of AC I and AC II faded away 20 days after drug withdrawal.

The drug-induced hypersensitization of AC I and AC II was fully reversible.

The second part of this work was oriented to the 2D electrophoretic resolution, immunoblot and proteomic analysis of post-nuclear supernatant (PNS) and Percoll-purified PM fractions, which were prepared from control (group –M10) and morphine-treated (group +M10) rats.
In the PNS, about 440 protein spots were recognized by silver staining and PDQuest analysis of gels in the both types of PNS. Staining by colloidal Coomassie blue G-250 indicated the total number of 200 spots. Ten of them exhibiting the largest morphine-induced change were selected, excised from the 2D gel and analyzed by MALDI-TOF MS/MS. The identified proteins were of cytoplasmic, cell membrane, endoplasmic reticulum and mitochondrial origin and 9 of them were significantly increased. Four of 9 up-regulated proteins were functionally related to the state of oxidative stress; the 2 proteins were related to the apoptotic cell death.

We could therefore conclude that the frontal brain cortex of rats exposed to increasing doses of morphine can not be regarded as adapted to morphine.

In the PM fraction analyzed by LC-MS/MS, the significantly up (↑)- or down (↓)-regulated proteins were of plasma membrane (Brain acid soluble protein 1, ↓2.1x; Gβ subunit 1, ↓2.0x), myelin membrane (MBP, ↓2.5x), cytoplasmic (Alpha-internexin, ↑5.2x; DPYL2, ↑4.9x; Ubiquitin hydrolase L1, ↓2.0x; 60S ribosomal protein L12, ↑2.7x; KCRB, ↓2.6x; SIRT2, ↑2.5x; Peroxiredoxin-2, ↑2.2x; Septin-11, ↑2.2x; TERA, ↑2.1x; SYUA, ↑2.0x; Coronin-1A, ↓5.4x) and mitochondrial (Glutamate dehydrogenase 1, ↑2.7x; SCOT1, ↑2.2x; Prohibitin, ↑2.2x; Aspartate aminotransferase, ↓2.2x) origin. Surprisingly, the immunoblot analysis of the same PM resolved by 2D electrophoresis indicated that the “active” pool of Gβ subunit (↓2.0x), represented just a minor fraction of the total signal of Gβ detected in 2D gels. The total signal of Gβ subunits was decreased 1.2x only; the major signal of Gβ subunits was unchanged. Likewise, the immunoblots of Gβ after resolution by 1D SDS-PAGE in 10% w/v acrylamide/0.26% w/v bis-acrylamide or 4-12% gradient gels (Invitrogen) indicated no change of this protein.

The “active”, minority pool of trimeric Gβ subunits responding to chronic morphine exposure of frontal rat brain cortex, was thus clearly recognized.

**Keywords:** morphine, long-term exposure, adenylyl cyclase isoforms (I-IX), frontal brain cortex, isolated plasma membranes, post-nuclear supernatant, proteomic analysis, oxidative stress, Gβ subunit