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Corrigendum

Corrigendum to "Exenatide (a GLP-1 agonist) expresses anti-inflammatory properties in cultured human monocytes/macrophages in A protein kinase A and B/Akt manner" [Pharmacol. Rep. 68 (2016) 329–337]

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An earlier corrigendum has been published but was found to be incorrect. This replaces it. In order to meet high standards of Pharmacological Reports and to improve the clarity of data presentation we wish to perform a corrigendum of methods of western blotting and graphical representation of Fig. 3. In details, western blotting methodology description was improved and representative blots of Fig. 3 were replaced. On the final steps of figure preparation the graphics technician made an incorrect attachment of blots into Fig. 3. The authors would like to apologize for any inconvenience caused and inform that all made corrections did not affect a scientific merit of the paper.

Protein extraction and western blotting

Cell extracts were prepared using a standard protocol previously described by Towbin et al. [14]. Monocyte cultures were washed from culture dishes with ice-cold PBS, and proteins were extracted with 100 μl of RIPA lysis buffer supplemented with protease inhibitors (Sigma-Aldrich, Warsaw, Poland). Cell lysates were incubated on ice, scraped off and shaken for 20 min on ice, centrifuged at 13,000 RPM (4 °C) and stored at −70 °C for further assays. Total protein concentrations in samples were determined spectrophotometrically according to Bradford [15]. Prior to assays, samples were diluted tenfold in water in order to avoid the interference between the reagents from the RIPA buffer and Bradford's reagent. Bovine serum albumin preparations (Fermentas, Vilnius, Lithuania) were used for calculation of the standard curve. Equal amounts of total protein (20 μg) mixed 1:1 with 2x sample buffer (25% glycerol, 2% sodium dodecyl sulfate, 0.02% bromophenol blue) were boiled for 6 min and loaded onto a 10% SDS-polyacrylamide gel. Electrophoresis was continued at 180 V (at constant voltage) until bromophenol blue dye reached the end of the gel. After electrophoresis, the stacking gels were cut-off and removed while resolving gels were further subjected to western blotting procedure in order to transfer proteins from polyacrylamide gels onto polyvinylidene fluoride (PVDF) membrane (Pall Poland Ltd., Warsaw, Poland). After the electrotransfer was finished, its proper efficacy as well as the protein load uniformity between the lanes was briefly controlled. To do it, membranes were left to dry for about five minutes and then soaked in a solution containing 20% methanol in water (v/v), which makes the lanes more opaque and thus visible if observed against the light. The nonspecific antibody binding was inhibited by incubation in TBST [20 mM Tris-buffered saline (pH 7.5) with 0.1% Tween 20] containing 5% non-fat dried milk for 1 h at room temperature (RT). Rabbit polyclonal antibodies against: iNOS, arg1 and MR were obtained from Sigma-Aldrich (Sigma-Aldrich, Poznań, Poland). As manufacturers declared, all antibodies show anti-human specificity. Antibodies were diluted in TBST containing 5% skim milk (1:500 dilution for iNOS and 1:1000 dilution for arg1 and MR). The membranes were incubated with the antibodies overnight at 4 °C, washed with TBST, incubated at RT for 60 min with the anti-rabbit, alkaline phosphatase (AP)-conjugated secondary antibodies diluted 1:1000 (Bio-Rad Laboratories Inc. Hercules, CA, USA) and washed twice with TBST for 5 min and once with TBS for 5 min [20 mM Tris-buffered saline (pH 7.8)]. In each assay, the colored precipitates were developed directly on the membrane using AP-chromogenic substrates (Bio-Rad Laboratories, Hercules, CA, USA). All of the membranes were photocopied and subjected to further analysis. The molecular masses for iNOS, arg1 and MR were confirmed according to the protein

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marker (PageRuler Unstained Protein Ladder, Fermentas, Vilnius, Lithuania) and the observed band sizes of all analyzed proteins were in concordance with those declared by the antibodies' supplier. If possible, for control of the proteins amount that was loaded in each lane, β-actin was detected simultaneously in each sample with the use of a 1:5000 dilution of the rabbit, anti-β-actin antibodies (Abcam Inc., Cambridge, MA, USA). If this approach was not possible, e.g. due to close proximity of the actin and a band of interest, then the same sample amount was analyzed simultaneously on a second gel and actin was detected and quantified. The integrated optical density (IOD) of signals was semiquantified using image-pro plus software and is expressed as the relative optical density (ROD), i.e. a ratio of the IOD for the tested proteins to the IOD for β-actin. Relative optical density values were subjected to statistical analysis.

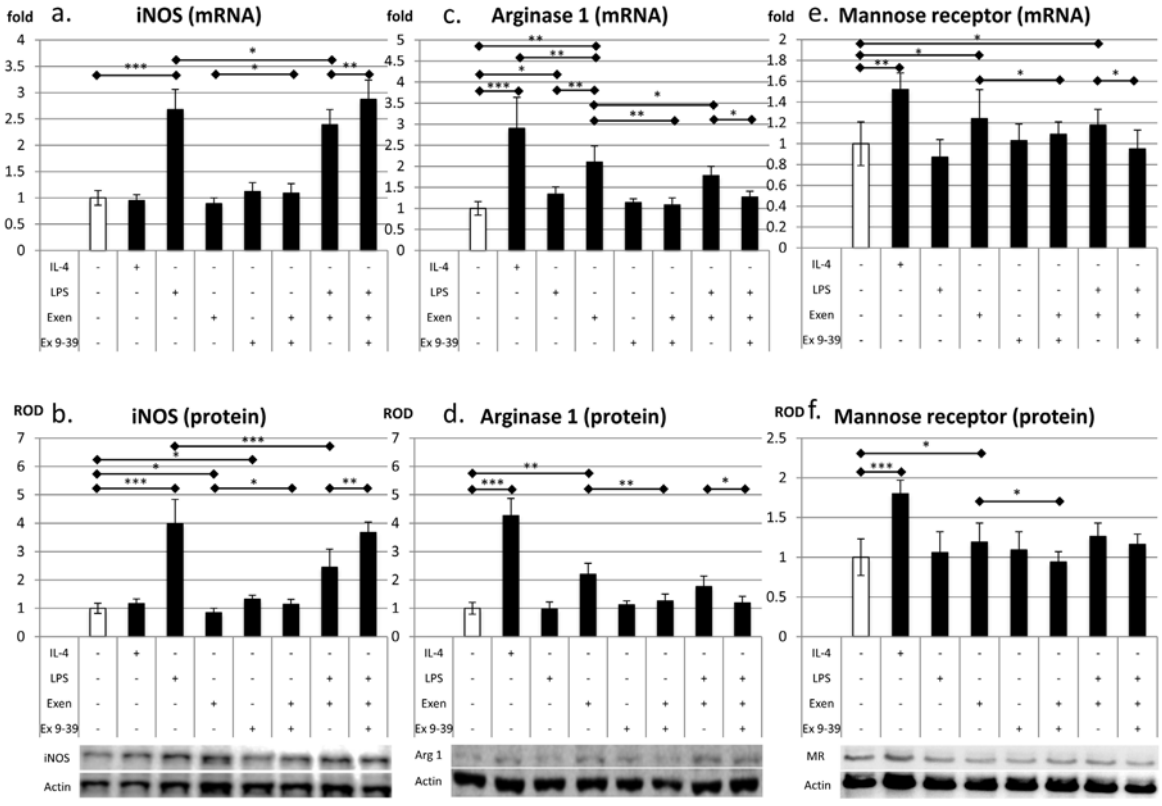

Fig. 3 Effects of exenatide, exendin 9-39, IL-4 and lipopolysaccharide (LPS) on mRNA and protein synthesis for iNOS (a and b), arginase 1 (c and d) and mannose receptor (e and f) in human monocytes/macrophages' cultures. The results represent the mean ± SD (n = 10); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Exen–exenatide, Ex 9-39–exendin 9-39.