Supplementary Material

Figure S1. Quantification of repetition of exon 2 in COT mRNA of WKY rat by PCR



Ratio of the two PCR products obtained by amplification from the two COT mRNA isoforms. Amplification was carried out with different cycle numbers. 1 μ l of cDNA obtained by RT from total kidney RNA was used as template. PCR amplification was performed with primers COT E1F and COT E3R. PCR conditions were as before except that 0.25 μ l of [α - ³²P] dCTP (10 μ Ci/ml 3000 mCi/mmol) were added to each reaction. Prior to loading, PCR reactions were mixed with formamide loading dye, heated at 94°C for 5 minutes and cooled on ice. PCR products were then separated on a 6% polyacrylamide gel. The radioactive signal was quantified with a phosphorimager, and log₁₀signal (arbitrary units) was plotted against the number of cycles. The signal at cycle 1 was calculated from each slope, corrected for the numbers of dCMP moieties incorporated on both strands, and used to calculate the proportion of the exon repetition isoform (13).