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Drug- and region-specific effects of protracted antidepressant and cocaine treatment on the content of melatonin MT₁ and MT₂ receptor mRNA in the mouse brain

Marta Imbesi, Tolga Uz, Sevim Yildiz, Ahmet D. Arslan, and Hari Manev

The Psychiatric Institute, Department of Psychiatry, University of Illinois at Chicago Chicago, Illinois 60612, USA

Abstract

Objectives—In the mammalian brain, G protein-coupled MT₁ and MT₂ melatonin receptors may be involved in Alzheimer's pathology, long-term potentiation, depression, and in the behavioral effects of psychoactive drugs. These drugs; e.g. antidepressants and drugs of abuse, are typically used over long periods of time and may alter neuroplasticity and gene expression. We hypothesized that such antidepressant- and cocaine-altered expression of melatonin receptor mRNA may occur in the hippocampus and striatum.

Methodology—Male C3H/HeJ mice were treated with the antidepressants fluoxetine, desipramine, and clomipramine, with the psychostimulant cocaine, and with a vehicle either a single time or once a day for 14 days. Brain samples were collected 24 h after the last injection and the content of MT₁ and MT₂ mRNA was assayed.

Results—A single drug injection did not alter the MT₁ and MT₂ mRNA content. In the hippocampus, protracted treatment with antidepressants increased the amount of MT₁ mRNA (with the exception of fluoxetine) but decreased MT₂ mRNA content; cocaine did not produce any alterations. In the striatum, antidepressants produced the opposite effect on MT₁ mRNA content; they decreased it. They did not significantly alter striatal MT₂ mRNA (we observed a nonsignificant trend to a decrease). Cocaine also decreased striatal MT₁ mRNA content without affecting MT₂ mRNA.

Conclusion—These results suggest that drug- and region-specific alterations of MT₁/MT₂ mRNA produced by protracted antidepressants and cocaine treatment may alter MT₁/MT₂ expression and contribute to long-term neuroplastic effects of these drugs.

Keywords

Melatonin receptors; fluoxetine; desipramine; clomipramine; hippocampus; striatum

Introduction

It has been suggested that the pineal hormone melatonin may be neuroprotective via the antioxidant actions of the melatonin molecule 1. However, the G protein-coupled metabotropic melatonin receptors MT₁ and MT₂ 2 are the primary mediators of the physiological actions of melatonin. The function of these receptors appears to be characterized by MT₁/MT₂ homo- and hetero-dimerization 3. These receptors are expressed

Correspondence should be addressed to: Prof. Hari Manev, M.D., Ph.D. The Psychiatric Institute, Department of Psychiatry University of Illinois at Chicago 1601 West Taylor Street, MC912 Chicago, IL 60612, USA Tel. 312-413-4558 Fax: 312-413-4569 E-mail: HManev@psych.uic.edu.

in the mammalian central nervous system (CNS) including humans 4-8, where they may play a role in CNS pathobiology and brain functioning, and in the mechanisms influencing the molecular and behavioral effects of psychoactive drugs.

For example, significant alterations in the intensity and distribution of MT₁ and MT₂ immunoreactivities were observed in postmortem brain from Alzheimer's patients; MT₁ immunoreactivity was significantly increased in Alzheimer's hippocampi 4, whereas MT₂ immunoreactivity in Alzheimer's hippocampi was decreased 5. Interestingly, transgenic mice deficient in MT₂ receptors demonstrated deficient hippocampal long-term potentiation (LTP) 9. Furthermore, when wild-type mice were tested in an elevated plus maze on two consecutive days, they showed shorter transfer latencies to enter a closed arm on the second day; this experience-dependent behavior did not occur in MT₂ knockouts 9. These results suggest that MT₂ receptors may participate in hippocampal synaptic plasticity and in memory processes.

A putative role for melatonin has also been proposed in mechanisms of depressive behaviors 10, 11 and in actions of antidepressant drugs 12. More recently, it was shown in a mouse model of forced swimming that the antidepressant-like behavioral effects of a melatonin receptor ligand require the expression of MT₂ but not MT₁ receptors 13.

MT₁ but not MT₂ receptors are required for nighttime inhibition of behavioral actions of cocaine; i.e., locomotor sensitization 14. Repeated injections of cocaine trigger locomotor sensitization only if delivered during the day (low melatonin) but not at night (high melatonin) 15. The nighttime inhibition of cocaine sensitization is reversed by pinealectomy, suggesting that the pineal gland, i.e., the main source of melatonin, may be responsible for this inhibition 16. In fact, it was shown that the nighttime inhibition of cocaine sensitization that occurs in wild-type mice is preserved in MT₂ knockout mice but is absent in MT₁ knockouts, suggesting that MT₁ receptors are required for inhibitory action of melatonin on the development of cocaine sensitization 14.

Objectives

CNS-acting drugs such as antidepressants and cocaine typically are used over prolonged periods of time when they cause significant and lasting alterations in neuroplasticity and in the expression of brain genes including those encoding a number of brain receptors. In this work, we hypothesized that such drug-altered expression of melatonin receptor mRNA may occur in brain regions involved in the actions of antidepressants and cocaine; i.e., the hippocampus and striatum. We tested this hypothesis in experiments with mice.

Methods

Animals and drug treatment

C3H/HeJ mice (6–8 per group), 8 weeks old and weighing 25–30 g were purchased from Jackson Laboratory (Bar Harbor, Maine). Animals were housed in groups of three and had free access to laboratory chow and water except during behavioral experiments. They were kept in a temperature-controlled room under conditions of 14 h light: 10 h dark cycle (lights on at 5 am; ZT00). Fluoxetine (10 mg/kg), desipramine (10 mg/kg), clomipramine (10 mg/kg), and cocaine hydrochloride (20 mg/kg) (Sigma Chemical Co., St. Louis, Missouri) were dissolved in sterile saline (0.9% NaCl) with 5% dimethyl sulfoxide (DMSO; Sigma Chemical Co.) and administered intraperitoneally (i.p.) in an injection volume of 50 µl/25 g body weight. Controls were injected with the same volume of vehicle. Mice were treated at 10 a.m. either for one day (single treatment) or for 14 days once a day (protracted treatment). The drug doses and injection schedules were chosen based on previous reports

15, 17, 18. The experimental protocol was approved by the Institutional Animal Care and Ethics Committee.

Reverse transcription - polymerase chain reaction (RT-PCR) assay

Striatal (i.e., caudate-putamen) and hippocampal tissue samples were collected 24 h after the last injection. They were excised from 1-mm-thick coronal brain slices as described elsewhere 19. Briefly, the caudate-putamen of the striatum was excised from the slices between the bregma 1.54 to -0.46mm, whereas the hippocampi were excised from the slices between the bregma -1.58 to -3.52mm. The total RNA was extracted using the chemical isolation method with TRIzol[®] reagent (Invitrogen, Carlsbad, California) following the manufacturer's instructions. To eliminate possible DNA contamination, RNA samples were treated with a DNase reagent, DNA-free[™] (Ambion, Inc., Austin, Texas). The yield of total RNA was determined by measuring the absorbance of an aliquot of the precipitated stock at a wavelength of 260/280 nm. Furthermore, to test possible DNA contamination, each sample was run by RT-PCR without adding the reverse transcriptase enzyme (see below).

Amplification primers for MT₁ (Gene Bank accession #U52222, forward (F): 5'-GTC AGC GCA TTT CTA ATG GGC TTG and reverse (R): 3'-GCT GTT GAA GTA CGC CAG GTA GTA), MT₂ (Gene Bank accession #.AY145850, F: 5'-TGA TAG CCT TGT ACC CTT ACC CAC and R: 3'-CCT GGA GCA CCA GTA CCC AGA TT), and for cyclophilin (Gene Bank accession #X52803, F: 5'-ATT TGG CTA TAA GGG TTC CTC and R: 3'-ACG CTC CAT GGC TTC CAC AAT) were purchased from Integrated DNA Technologies, Inc. (Coralville, IA). To allow co-amplification of either MT₁ or MT₂ mRNA with the more abundant cyclophilin mRNA, pilot studies were conducted to determine optimal relative primer concentrations and cycle numbers whereby the PCR would still be within the exponential (i.e., linear) range for both transcripts. The assay was performed as described elsewhere 7. Briefly, total RNA (0.5 µg of starting amount) was denatured and then reverse transcribed. The cDNA aliquots containing reverse-transcribed material and specific primer pairs for either MT₁ or MT₂ along with cyclophilin (the ratio to cyclophilin is 1:10) were amplified with Hot Tub DNA polymerase (Amersham, Piscataway, NJ) in a thermal cycler (9600, Perkin Elmer, Wellesley, MA). The PCR mixture was amplified for 34 cycles with denaturation (94°C, 15 s), annealing (65°C for MT₁ and 60°C for MT₂, 30 s), and elongation (72°C, 30 s). The reaction was terminated with a 5-min final elongation step. Trace amounts of [³²P]dCTP (0.5 µCi/sample; Amersham, Piscataway, NJ) were included during the PCR step for subsequent quantification. For each sample, the ethidium bromide-stained bands for MT₁, MT₂ and cyclophilin were excised from the electrophoresis gels and the radioactivity was counted 7. Results were calculated as the ratio of either MT₁ or MT₂ to cyclophilin (mean ± S.E.M.).

Statistical analysis

A one-way between-group analysis of variance was followed by the 2-sided Dunnett test for a multiple comparison with control (SPSS 12.0 for Windows) and the mean difference was accepted as significant at the 0.05 level.

Results

Protracted administration of antidepressants but not cocaine produces differential effects on MT₁ vs. MT₂ expression in the hippocampus

Twenty four hours after the fourteenth injection of each of the three antidepressants we used, the amount of hippocampal MT₂ mRNA was reduced in comparison with vehicle-treated controls (Fig. 1). In contrast, the amount of MT₁ mRNA was increased by antidepressants (except for fluoxetine). The same schedule of cocaine administration failed to alter hippocampal MT₁ and MT₂ mRNA levels (Fig. 1).

Protracted administration of antidepressants and cocaine decreases MT₁ expression in the striatum

The protracted (i.e., 14-day) treatment with antidepressants did not significantly alter the striatal MT₂ mRNA content (Fig. 2) although, similar to hippocampal findings, it did produce a trend to a decrease. Unlike in the hippocampus, the antidepressants decreased and not increased the striatal content of MT₁ mRNA. Protracted treatment with cocaine also decreased striatal MT₁ but not MT₂ mRNA levels (Fig. 2).

A single injection of antidepressants and cocaine fails to alter MT₁ and MT₂ expression

In contrast to the altered MT₁/MT₂ expression observed after protracted drug treatment, a single injection of antidepressants and cocaine did not significantly alter MT₁ and MT₂ mRNA levels either in the hippocampus (Fig. 3) or the striatum (Fig. 4).

Discussion

In this work, we found evidence that the CNS content of melatonin receptor mRNA is significantly modified by prolonged treatment with antidepressants and cocaine. Interestingly, the effects of these drugs on MT₁/MT₂ mRNAs were brain region-specific. For example, the effect of antidepressants on MT₁ expression was opposite in the hippocampus vs. the striatum. The expression of the melatonin receptor mRNA is reduced/downregulated upon exposure to melatonin 20. Thus, it is unlikely that the differential region-specific effect of antidepressants on MT₁ mRNA levels; i.e., a concomitant up-regulation (hippocampus) and down-regulation (striatum), is mediated by antidepressant-altered levels of circulating melatonin. In fact, the antidepressants we used in this study apparently do not affect melatonin levels in-vivo 21. Hence, the regional differences we observed may relate to region-specific presence of the primary (e.g., serotonin, noradrenaline and dopamine transporters) or secondary (e.g., clock genes) 18 targets for the CNS actions of drugs used in our study.

Also obvious was the difference between the effects of antidepressants and cocaine on MT₂ expression; cocaine did not alter this expression whereas all three antidepressants significantly reduced MT₂ expression in the hippocampus and caused a non-significant trend to MT₂ mRNA decreases in the striatum. The similar effect of all three antidepressants on melatonin receptor expression is rather surprising because the drugs we used operate via different primary mechanisms of action. Fluoxetine is a predominant serotonin reuptake inhibitor, desipramine is a tricyclic predominantly noradrenaline reuptake inhibitor, and clomipramine is a tricyclic serotonin/noradrenaline reuptake inhibitor. It is possible that a common second or third messenger intracellular mechanism, which is triggered by protracted but not single drug application that may be responsible for the common therapeutic, i.e., antidepressant, activity of these drugs, also regulates the expression of melatonin receptors.

Interestingly, the effect of antidepressants on hippocampal MT₁/MT₂ mRNA content is similar to the alterations in hippocampal MT₁/MT₂ protein content observed in Alzheimer's disease 4-5. In both cases, MT₁ receptors appear to be up-regulated and MT₂ receptors are down-regulated. It was shown that melatonin can inhibit/impair hippocampal LTP (a model for learning/memory) and that this impairment is reduced by a MT₂ deficiency 22. Hence, it is possible that a relative shift toward reduced hippocampal MT₂ receptors could be beneficial. If so, the findings in postmortem Alzheimer's brain could reflect a compensatory mechanism. Further studies are needed to investigate whether mRNA changes observed in our study are accompanied by corresponding alterations in the content of melatonin receptor proteins.

If drug-induced changes in hippocampal and striatal expression of melatonin receptor mRNA are accompanied by similar changes in receptor protein content and also occur in humans, our study may point to several clinically important implications. For example, depression is often associated with sleep disturbances and a new MT₁/MT₂ agonist, ramelteon, has recently been introduced as a therapy for insomnia 23. Since it is likely that ramelteon will be used in combination with antidepressants, clinical studies may be warranted to clarify whether antidepressant treatment would interfere with the therapeutic effects of ramelteon. Similarly, it might be worth exploring whether ramelteon should be used in combination with antidepressants. If antidepressant effects include changes in the CNS expression of melatonin receptors, ramelteon might cause unforeseen interactions with antidepressant drugs, both beneficial and detrimental to antidepressant therapy. Finally, considering the participation of melatonin receptors in the long-term effects of cocaine 14, a possible role for melatonin receptor agonists in drug abuse should also be considered.

Acknowledgments

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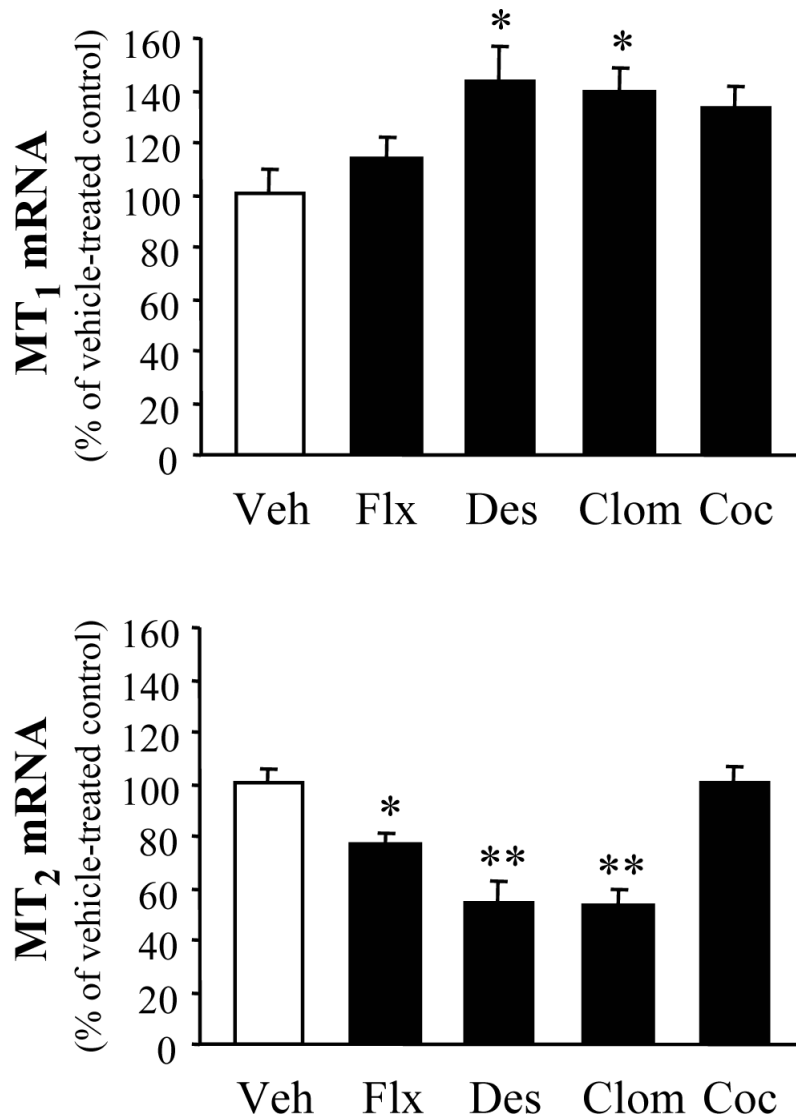


Figure 1. Effects of protracted antidepressant and cocaine treatment on hippocampal melatonin receptor mRNA levels. Samples were collected 24 h after the 14th injection of vehicle (Veh), fluoxetine (Flx), desipramine (Des), clomipramine (Clom), and cocaine (Coc). Results were calculated as the ratio of either MT₁ or MT₂ to cyclophilin. The ratios in vehicle-treated controls were: MT₁/cyclophilin = 0.22±0.02; MT₂/cyclophilin = 0.30±0.02. Results are expressed as a percentage of the corresponding vehicle-treated controls (mean ± S.E.M.). *P<0.05, **P<0.001 vs. Veh (Dunnett test).

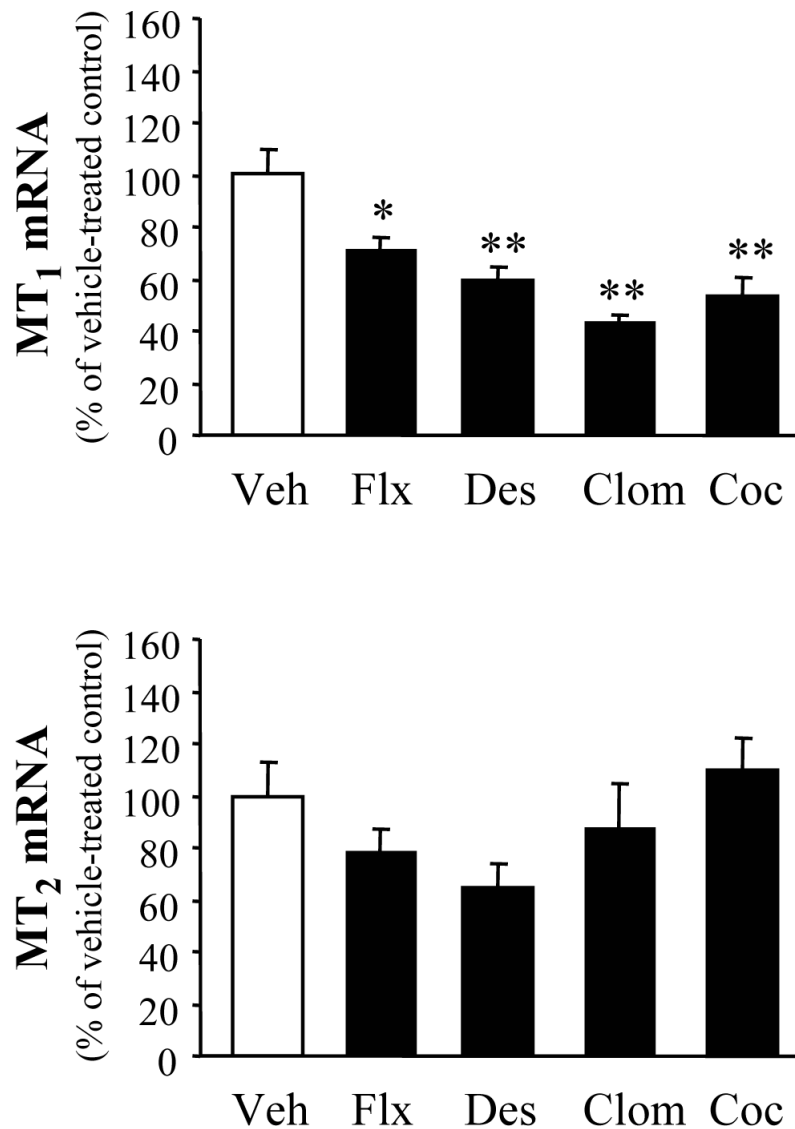


Figure 2. Effects of protracted antidepressant and cocaine treatment on striatal melatonin receptor mRNA levels. Samples were collected 24 h after the 14th injection of vehicle (Veh), fluoxetine (Flx), desipramine (Des), clomipramine (Clom), and cocaine (Coc). Results were calculated as the ratio of either MT₁ or MT₂ to cyclophilin. The ratios in vehicle-treated controls were: MT₁/cyclophilin = 0.37 ± 0.03 ; MT₂/cyclophilin = 0.30 ± 0.04 . Results are expressed as a percentage of the corresponding vehicle-treated controls (mean \pm S.E.M.). *P<0.05, **P<0.01 vs. Veh (Dunnett test).

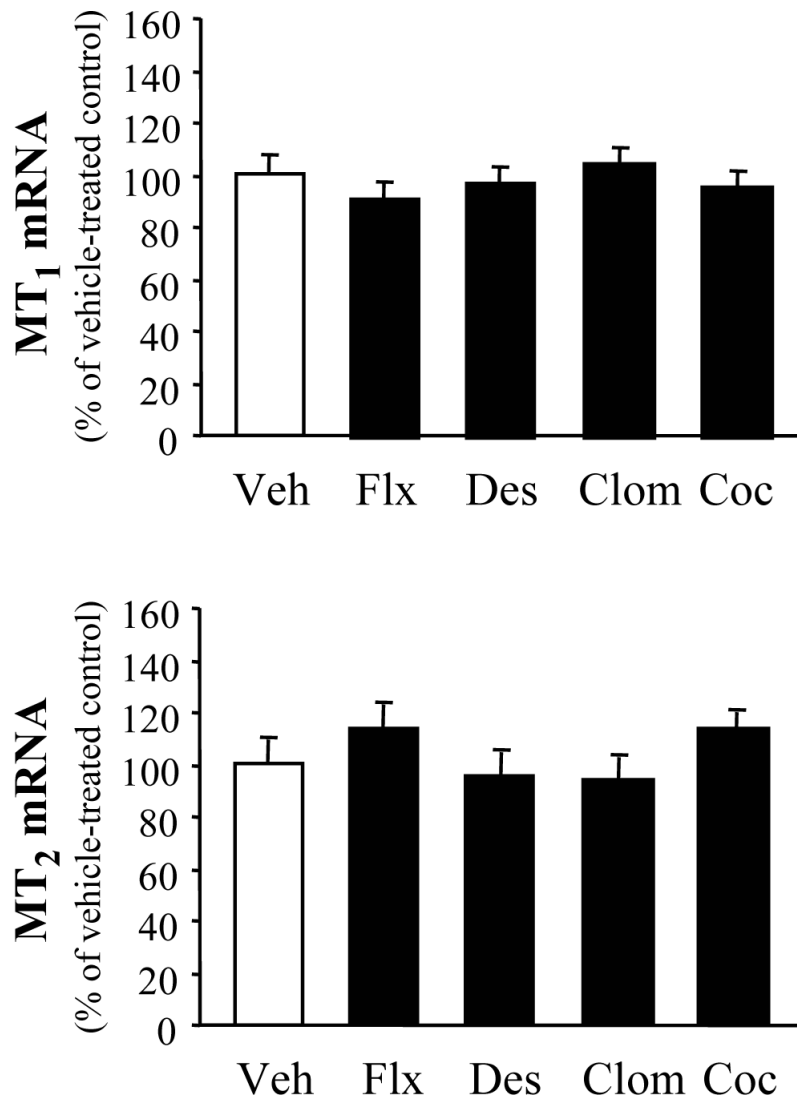


Figure 3.

Hippocampal melatonin receptor mRNA levels after single injections of antidepressant and cocaine. Mice (6–8 per group) were injected with vehicle (Veh), fluoxetine (Flx), desipramine (Des), clomipramine (Clom), and cocaine (Coc) 24 h prior to sample collection. Results (mean \pm S.E.M.) were calculated as MT_1 /cyclophilin and MT_2 /cyclophilin mRNA ratios, and are expressed as a percentage of the corresponding vehicle-treated group. No significant differences were observed between drug- vs. vehicle-treated groups; $MT_1 = [F(4, 30) = 0.345, P = 0.845]$, $MT_2 = [F(4, 34) = 0.502, P = 0.734]$.

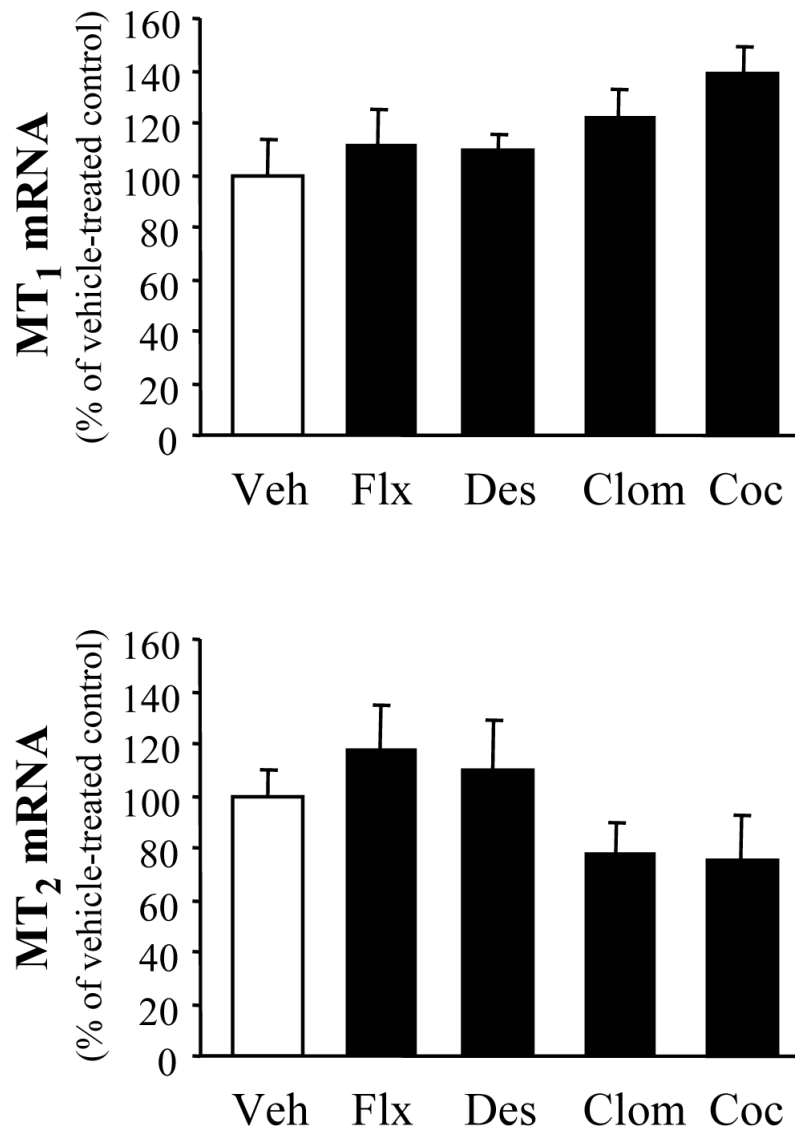


Figure 4. Striatal melatonin receptor mRNA levels after single injections of antidepressant and cocaine. Mice (6–8 per group) were injected with vehicle (Veh), fluoxetine (Flx), desipramine (Des), clomipramine (Clom), and cocaine (Coc) 24 h prior to sample collection. Results (mean \pm S.E.M.) were calculated as MT₁/cyclophilin and MT₂/cyclophilin mRNA ratios, and are expressed as a percentage of the corresponding vehicle-treated group. No significant differences were observed between drug- vs. vehicle-treated groups; MT₁ = [F (4, 29) = 1.113, P = 0.370], MT₂ = [F (4, 30) = 1.782, P = 0.159].