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CHRONIC 3,4-METHYLENEDIOXYMETHAMPHETAMINE ADMINISTRATION DECREASES GLUCOCORTICOID AND MINERALOCORTICOID RECEPTOR, BUT INCREASES 5-HYDROXYTRYPTAMINE$_{1C}$ RECEPTOR GENE EXPRESSION IN THE RAT HIPPOCAMPUS

J. L. W. YAU,*† P. A. T. KELLY,‡ J. SHARKEY‡ and J. R. SECKL*

University of Edinburgh, Departments of *Medicine and ‡Clinical Neurosciences, Western General Hospital, Edinburgh EH4 2XU, U.K.

Abstract—Both glucocorticoids and serotonin have been implicated in the regulation of mood and neuroendocrine control. In this study we have examined the effects of the psychomotor stimulant, 3,4-methylenedioxymethamphetamine on corticosteroid and 5-hydroxytryptamine receptor subtype gene expression within the hippocampal formation using *in situ* hybridization histochemistry. Animals were injected subcutaneously with 3,4-methylenedioxymethamphetamine (20 mg/kg) twice daily for four days. Two weeks following this dosage regimen, shown to markedly reduce 5-hydroxytryptamine terminals, both glucocorticoid receptor and mineralocorticoid receptor messenger RNA expression were significantly decreased (30–47% fall) in the granule cells of the dentate gyrus and pyramidal cells of CA1–CA4 fields of Ammon's horn, but not in parietal cortex neurons. In the same rats, 5-hydroxytryptamine$_{1C}$ receptor messenger RNA expression was significantly increased in CA3 pyramidal neurons (133% rise), but neither 5-hydroxytryptamine$_{1A}$ or 5-hydroxytryptamine$_2$ receptor messenger RNA levels were altered in any dorsal hippocampal subfield. 3,4-Methylenedioxymethamphetamine treatment was associated with modest hypersecretion of coricosterone during the diurnal nadir, without other peripheral evidence of chronic glucocorticoid excess (unchanged thymic and adrenal weights and corticosterone-binding globulin levels).

These results emphasize the importance of the serotonergic innervation in maintaining hippocampal corticosteroid receptor gene expression. It is suggested that 5-hydroxytryptamine$_{1C}$ receptors may be involved in mediating the effects of serotonin on hippocampal glucocorticoid receptor and mineralocorticoid receptor expression and perhaps mood.

The ring-substituted amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA) is a widely abused recreational drug which causes selective depletion of 5-hydroxytryptamine (5-HT) and subsequent degeneration of serotonergic terminals in rat and primate brain.[43,51,52] Central 5-HT systems are thought to underpin a number of critical functions of the brain, including control of mood, specific behaviours and neuroendocrine systems.[17,19,35] The increasing abuse of MDMA has therefore prompted serious concern over the neuropsychiatric and neuroendocrine sequellae of human exposure[45,47,50] and, indeed, affective and psychotic disturbances are being increasingly recognized in association with MDMA abuse.[8,22]

All subfields of the hippocampus receive extensive innervation from 5-HT cell bodies in the brain stem raphe nuclei.[44] At the hippocampus, 5-HT is thought to influence mood and also interact with the brain-pituitary-adrenal axis.[60] The hippocampus contains the greatest density of corticosteroid receptors in the brain, which are of two types, mineralocorticoid (MR, type I) and glucocorticoid (GR, type II).[33] Lesions of the hippocampus or manipulations which reduce hippocampal corticosteroid receptor expression result in elevated basal corticosterone levels and attenuated glucocorticoid suppression of hypothalamic-pituitary-adrenal axis activity.[26] Similar neuroendocrine changes are also reported in depressive disorders in which defective 5-HT neurotransmission (in the cortex and limbic system) has been implicated.[35] Clearly, therefore, any interaction in the hippocampus between the 5-HT innervation and corticosteroid receptor bearing cells is of considerable interest.

In vitro, 5-HT increases glucocorticoid receptor expression in primary cultures of fetal hippocampal neurons, where its actions are mediated by ketanserin-sensitive 5-HT$_2$ (or possibly 5-HT$_{1C}$) receptors.[39] *In vivo*, electrophysiological evidence indicates a direct interaction between 5-HT and corticosteroid-responsive hippocampal neurons.[4,27] Furthermore, lesions to central 5-HT pathways, using the neurotoxin

†To whom correspondence should be addressed.

Abbreviations: CBG, corticosterone-binding globulin; 5,7-DHT, 5,7-dihydroxytryptamine; EDTA, ethylenediaminetetra-acetate; GR, glucocorticoid receptor; 5-HT, 5-hydroxytryptamine; MDMA, 3,4-methylenedioxymethamphetamine; MR, mineralocorticoid receptor; SSC, saline sodium citrate.

5,7-dihydroxytryptamine (5,7-DHT), decrease nuclear corticosterone binding sites in hippocampal extracts[60] and attenuate the expression of mRNAs encoding both MR and GR in specific subregions of the hippocampus.[57] If these *in vivo* effects are related to attenuated 5-HT neurotransmission rather than effects of 5,7-DHT on other systems, then MDMA, which we have previously demonstrated to affect hippocampal function (at least as assessed by alterations in metabolic activity[59]) would be predicted to reduce hippocampal corticosteroid receptor gene expression. We have therefore now examined the effects of MDMA on hippocampal MR and GR mRNA expression. Furthermore, since elevation of basal corticosterone levels following MDMA is mediated via a ketanserin-sensitive 5-HT_2 or (5-HT_{1C}) receptor mechanism[41] and the 5-HT receptor types responsive to alterations in activity of the 5-HT innervation to the hippocampus are poorly defined, we have examined the effects of MDMA on the expression of genes encoding the major hippocampal 5-HT receptor subtypes, 5-HT_{1A}, 5-HT_{1C} and 5-HT_2.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (150–175 g) were maintained under controlled lighting (lights on 07.00–19.00 h) and temperature (20°C) and had access to food and water *ad libitum*. Rats were injected subcutaneously with either saline (1 ml/kg) or MDMA (20 mg/kg as the free base; Sigma Chemicals Ltd) twice a day for four consecutive days and then killed by decapitation (between 09.00 and 11.00 h) 14 days after the final injection. Trunk blood samples were taken for determination of plasma corticosterone and corticosterone-binding globulin (CBG) and the adrenal and thymus glands were weighed. The brains were rapidly removed and frozen on powdered dry-ice. Cryostat sections (20 μm) at the level of the anterior dorsal hippocampus were cut in the coronal plane, thaw-mounted on to gelatine-subbed, poly-L-lysine-coated slides and stored at −85°C prior to *in situ* hybridization. Adjacent sections to those used for *in situ* hybridization were thaw-mounted on to gelatine coated slides for subsequent [^{3}H]paroxetine binding autoradiography.

In situ *hybridization*

In situ hybridization was carried out as described previously.[57] Brain sections were postfixed in 4% paraformaldehyde, washed twice in 2 × saline sodium citrate (SSC) and hybridized overnight at 50°C using [^{35}S]UTP-labelled cRNA antisense probes transcribed *in vitro* from plasmid vectors containing the appropriate cDNA insert. MR cRNA was transcribed from a 513 bp EcoR1 fragment of a rat MR cDNA (representing the steroid binding domain and part of the 3′-untranslated region), GR cRNA from a 674 bp PstI-EcoR1 fragment of rat GR cDNA (steroid binding domain), 5-HT_{1A} receptor cRNA from a 910 bp BalI-PvuII fragment of the rat 5-HT_{1A} receptor cDNA[1] (second transmembrane domain to the cytoplasmic loop between transmembrane domains VI and VII), 5-HT_{1C} receptor cRNA from a 401 bp EcoR1-Sst1 fragment of the rat 5-HT_{1C} receptor cDNA[28] (5′-untranslated region), and 5-HT_2 receptor cRNA from a 1.1 kb Hpa1-EcoR1 fragment of the rat 5-HT_2 receptor cDNA[48] (half of the entire cDNA sequence including the third intracytoplasmic loop). Probes were denatured and added at a final concentration of 10×10^6 c.p.m./ml (MR, GR and 5-HT_2) and 20×10^6 c.p.m./ml (5-HT_{1A} and 5-HT_{1C}) to hybridization buffer. For all three 5-HT receptor cRNA probes, an additional prehybridization step involving the addition of 200 μl of prehybridization buffer (hybridization buffer omitting the dextran sulphate) per slide and incubation at 50°C for 2 h was included as this was found in preliminary experiments to enhance the hybridization signal/noise ratio. Following hybridization, sections were treated with RNase A (30 μg/ml, 45 min at 37°C) and washed to a final stringency of 0.1 × saline sodium citrate (SSC) at 60°C. Slides were dehydrated, dipped in photographic emulsion (Ilford, U.K.) and exposed at 4°C for 21 days before being developed and counterstained with haematoxylin and eosin. Control sections were hybridized with identically labelled sense cRNA probes.

Data analysis

Hybridization signal within hippocampal subregions was assessed by computer-assisted grain counting using an image analysis system (Seescan plc, Cambridge, U.K.). Silver grains were counted within high power microscopic fields under brightfield illumination. Each microscopic field covered approximately two adjacent neurons within each hippocampal subfield, except in the dentate gyrus where it was difficult to define cell boundaries. For each animal the mean grain counts over eight to 12 fields/subregion after subtraction of background (counted over areas of white matter, e.g. corpus callosum) were taken. Microscopic fields covering the area and immediate surrounding of a single neuron were used for 5-HT_2 and 5-HT_{1C} receptor mRNA expression due to the scattered nature of silver grains over these highly expressing neurons. Data were assessed by ANOVA followed by paired or unpaired Students t-tests, as appropriate. Significance was set at $P < 0.05$. Values are means ± S.E.M.

Binding studies

The density of 5-HT uptake sites in both control and MDMA-treated rat brains was determined by [^{3}H]paroxetine binding autoradiography performed in accordance with previously published protocols. Sections were incubated for 2 h in buffer (50 mM Tris-HCl; 120 mM NaCl; 5 mM KC; pH 7.7 at 23°C) containing a saturating concentration (250 pM) of [^{3}H]paroxetine (specific activity, 23.1 Ci/mmol; New England Nuclear).[16] Nonspecific binding was defined in adjacent sections by incubating with [^{3}H]paroxetine in the presence of 4 μM citalopram. At the end of the incubation period, the sections were washed in buffer, dipped through deionized water and rapidly dried under a stream of cold air. The sections were apposed to X-ray film (Amersham Hyperfilm) together with a set of precalibrated tritium-containing standards and stored in light-tight cassettes at −70°C for four to six weeks.

Analysis of [^{3}H]paroxetine binding autoradiograms was performed using a computer-based image analysis system (Quantimet 970; Cambridge Instruments, U.K.). For each hippocampal subfield of interest, optical density measurements were made bilaterally from six consecutive autoradiographic images, the local tissue tracer concentrations were derived by comparison of the mean optical density of the area of interest with the optical densities of the standards. Specific binding was determined by the subtraction of nonspecific binding images (incubated in the presence of citalopram) from the total binding found when sections were incubated with [^{3}H]paroxetine alone. Data were assessed using Students t-test for grouped data with acceptable levels of significance set at $P < 0.05$.

Corticosterone binding globulin and corticosterone assay

Plasma CBG levels was measured as previously described.[58] In brief, endogenous steroids were removed from plasma by passing the sample (20 μl aliquot of plasma)

through a 10 × 1 cm Sephadex LH-20 (Pharmacia Fine Chemicals) column and diluted 50:1 with TEDGM buffer (30 mM Tris, pH adjusted to 7.4 with HCl, 1 mM EDTA, 10 mM sodium molybdate, 1 mM dithiothreitol and 10% glycerol). Aliquots (225 μl) of diluted plasma were incubated in buffer (150 μl) containing a saturating 80 nM concentration of [^{3}H]corticosterone (specific activity 88 Ci mmol, Amersham, England) for 90 min at 2–4°C. Nonspecific binding was defined in parallel incubations by a 200-fold excess of cold corticosterone. Following incubation, bound and unbound steroids were separated on Sephadex LH-20 column, equilibrated with TEDGM. After 30 min, columns were eluted with 500 μl TEGM buffer (10 mM Tris, 2 mM EDTA, 10 mM sodium molybdate and 2.3 mM β-mercaptoethanol) into scintillation vials and counted. Protein content was determined by the method of Bradford[9] and the results expressed as pmol [^{3}H]corticosterone bound/mg protein.

Plasma corticosterone was measured using a previously described radioimmunoassay[2] with a highly specific corticosterone antiserum [raised in rabbits against corticosterone-3-(O-carboxymethyl) oximine-BSA immunogen and gamma-labelled corticosterone of high specific activity] and [^{125}I]corticosterone [in-house synthesis by coupling corticosterone-3-(O-carboxymethyl) oxime to a previously radioiodinated histamine using the method described by Al-Dujaili and Edwards[3] for aldosterone].

RESULTS

Paroxetine binding

The distribution of [^{3}H]paroxetine-labelled 5-HT uptake sites in hippocampal subfields is shown in Table 1. In control animals, the highest densities of [^{3}H]paroxetine binding were found in the CA2 and CA3 fields of Ammon's horn. Repeated treatment with MDMA, resulted in profound reductions in [^{3}H]paroxetine binding densities throughout the hippocampus, but these were most marked in the CA2 and CA3 fields (−92 and −87%, respectively). Similar effects were also measured in parietal cortex (−77%).

Organ weights, corticosterone binding globulin and corticosterone measurements

The weights ($n = 18$/group) of the adrenal glands and thymus from MDMA-treated rats were not significantly different from those in controls (Table 2). MDMA did not affect plasma CBG levels, whereas basal plasma corticosterone was significantly increased (102% rise, $P < 0.05$) in MDMA-treated rats compared to controls (Table 2).

Table 1. Effects of 3,4-methylenedioxymethamphetamine exposure upon [^{3}H]paroxetine-labelled 5-hydroxytroptamine uptake sites in the rat brain

Brain region	Saline pretreated	MDMA pretreated
Hippocampus		
Dentate gyrus	80 ± 16	45 ± 11*
CA1	72 ± 8	25 ± 5*
CA2	150 ± 13	12 ± 6*
CA3	166 ± 15	22 ± 7*
Parietal cortex	200 ± 12	45 ± 9*

Data are presented as mean binding density (fmol/mg tissue) ± S.E.M. $n = 5$/group. *, significantly different from control ($P < 0.05$).

Table 2. Effect of 3,4-methylenedioxymethamphetamine treatment on adrenal and thymus weights, expressed relative to total body weight, and corticosterone-binding globulin and corticosterone levels

	Saline pretreated	MDMA pretreated
Adrenal weight (mg/100 g BW)	14.32 ± 0.38	14.10 ± 0.38
Thymus weight (mg/100 g BW)	262 ± 6.0	277 ± 5.0
CBG (pmol/mg protein)	16.30 ± 1.21	15.65 ± 1.56
Corticosterone (μg/dl)	3.57 ± 0.78	7.24 ± 1.17*

Results represent the mean ± S.E.M. ($n = 18$/group). *Significantly different from control ($P < 0.05$). BW, body weight.

Mineralocorticoid receptor and glucocorticoid receptor mRNA expression

GR mRNA was highly expressed in granule cells of the dentate gyrus and in pyramidal neurons of CA1 and CA2, but expression was lower in CA3 and CA4, whereas MR and mRNA was highly and approximately equally expressed in all hippocampal subfields, as previously described.[57] MDMA treatment significantly decreased both MR and GR mRNA expression in all hippocampal regions (Fig. 1). GR mRNA expression was decreased by between 39% and 46% ($P < 0.001$) (e.g., Fig. 2A), MR by between 30 and 41% ($P < 0.001$) (Fig. 2B), depending on the hippocampal subfield. Neither MR nor GR mRNA expression were altered in the overlying retrosplenial granular layer of the parietal cortex by MDMA treatment despite the reduction in [^{3}H]paroxetine binding in this region (see Table 1). Repeated MDMA exposure did not appear to cause any obvious hippocampal neuronal loss or degeneration, as assessed by histological inspection.

5-Hydroxytryptamine receptor subtype mRNA expression

In control rats, 5-HT$_{1A}$ receptor mRNA was highly expressed in the granule cell layer of the dentate gyrus and the pyramidal cell layer of Ammon's horn, particularly in CA3 and CA4, in good agreement with previous studies.[11,12,46] MDMA treatment had no significant effect on 5-HT$_{1A}$ receptor expression in any hippocampal subfield in the dorsal hippocampus (Fig. 3A).

The highest level of expression of 5-HT$_{1C}$ receptor mRNA was localised in choroid plexus epithelial cells as shown by other studies.[25,36,40] In the anterior dorsal hippocampus, 5-HT$_{1C}$ receptor mRNA expression was low in the dentate gyrus and CA1, whereas some heavily labelled neurons were observed in the strata radiatum and oriens of CA2 and CA3. Within the cerebral cortex, few neurons expressed 5-HT$_{1C}$ recep-

tor mRNA. However, prominent hybridisation was detected in the retrosplenial granular layer of the parietal cortex. Chronic MDMA treatment significantly increased 5-HT_{1C} receptor mRNA levels in the strata radiatum and oriens of the CA3 hippocampal subregion (133% increase over control, $P < 0.01$; Fig. 2C) but had no significant effect on 5-HT_{1C} receptor expression in dentate gyrus, CA2 or the parietal cortex (Fig. 3B).

5-HT_2 receptor mRNA expression also varied greatly between adjacent neurons within a hippocampal subfield, with some neurons being strongly labelled and others unlabelled. High expression of 5-HT_2 receptor mRNA was observed in layers 2–5 of the parietal cortex as previously shown.[37] Scattered, highly expressing cells in the hilus dentate gyrus and pyramidal cells in CA2 and CA3 of the anterior portion of the dorsal hippocampus were also found. No hybridization of the 5-HT_2 receptor cRNA was seen in the choroid plexus, indicating no cross hybridization of this probe with 5-HT_{1C} receptor mRNA. MDMA treatment did not alter the level of 5-HT_2 receptor mRNA expression in any hippocampal subfield or the parietal cortex (Fig. 3C). Control sections hybridized with sense probes showed no labelling above background (data not shown, but see for example Ref. 57).

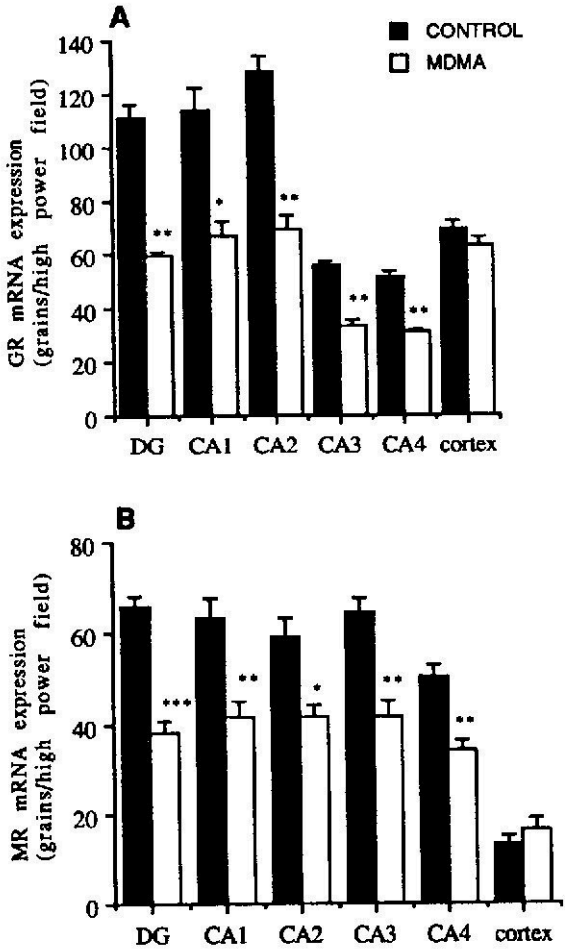

Fig. 1. Effect of chronic MDMA treatment on (A) glucocorticoid receptor (GR) and (B) mineralocorticoid receptor (MR) mRNA expression in hippocampal subregions and parietal cortex. Sprague–Dawley rats were given 0.9% saline or MDMA (20 mg/kg, s.c. twice daily for four days.) Rats were sacrified 14 days after the last injection. Following hybridisation of brain sections with GR and MR riboprobes as described in Experimental Procedures, slides were dipped in photographic emulsion, exposed for three to four weeks then developed and counterstained with haemotoxylin and eosin. DG, dentate gyrus. Results represent the mean ± S.E.M. ($n = 5$/group). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to control.

DISCUSSION

3,4-*methylenedioxymethamphetamine neurotoxicity: effects on mineralocorticoid receptor and glucocorticoid receptor mRNA expression*

MDMA has been shown to be neurotoxic in the rat with long-term effects including marked decreases in the concentrations of 5-HT and 5-hydroxyindoleacetic acid and in the activity of tryptophan hydroxylase, the rate limiting enzyme of 5-HT synthesis.[7,15,43] In addition, repeated systemic administration of MDMA results in the destruction of 5-HT terminals as shown by a reduction in the number of uptake sites for 5-HT.[7,43] Our dosage regimen for MDMA, previously shown to decrease rat hippocampal 5-HT content by 70%[15] produced the expected loss of 5-HT uptake sites, as evidenced by the marked (44–92%) decrease in [^{3}H]paroxetine binding sites in hippocampus and parietal cortex. MDMA treatment was also associated with a marked decrease in the expression of MR and GR mRNAs in all hippocampal subregions. Taken together with our previous results showing decreased expression of hippocampal MR and GR mRNAs following administration of 5,7-dihydroxytryptamine,[57] these data provide evidence for an important role for 5-HT in the maintenance of corticosteroid receptor biosynthesis in the limbic system. This regulation is apparently specific to the hippocampus as corticosteroid receptor gene expression was not altered in the overlying parietal cortex, although neurons here also express both GR and MR mRNAs,[13,24] the region receives 5-HT projections[44] and 5-HT uptake sites were depleted by MDMA to an extent similar to the hippocampus. Furthermore, although 5-HT fibres vary in their sensitivity to MDMA neurotoxicity,[43] projections to both hippocampus and parietal cortex are affected by MDMA, and the hippocampus, at least, shows persisting functional changes following a similar administration protocol.[59] It therefore seems unlikely that differential sensitivity of projections to MDMA underlies the selectivity of effects on the hippocampus.

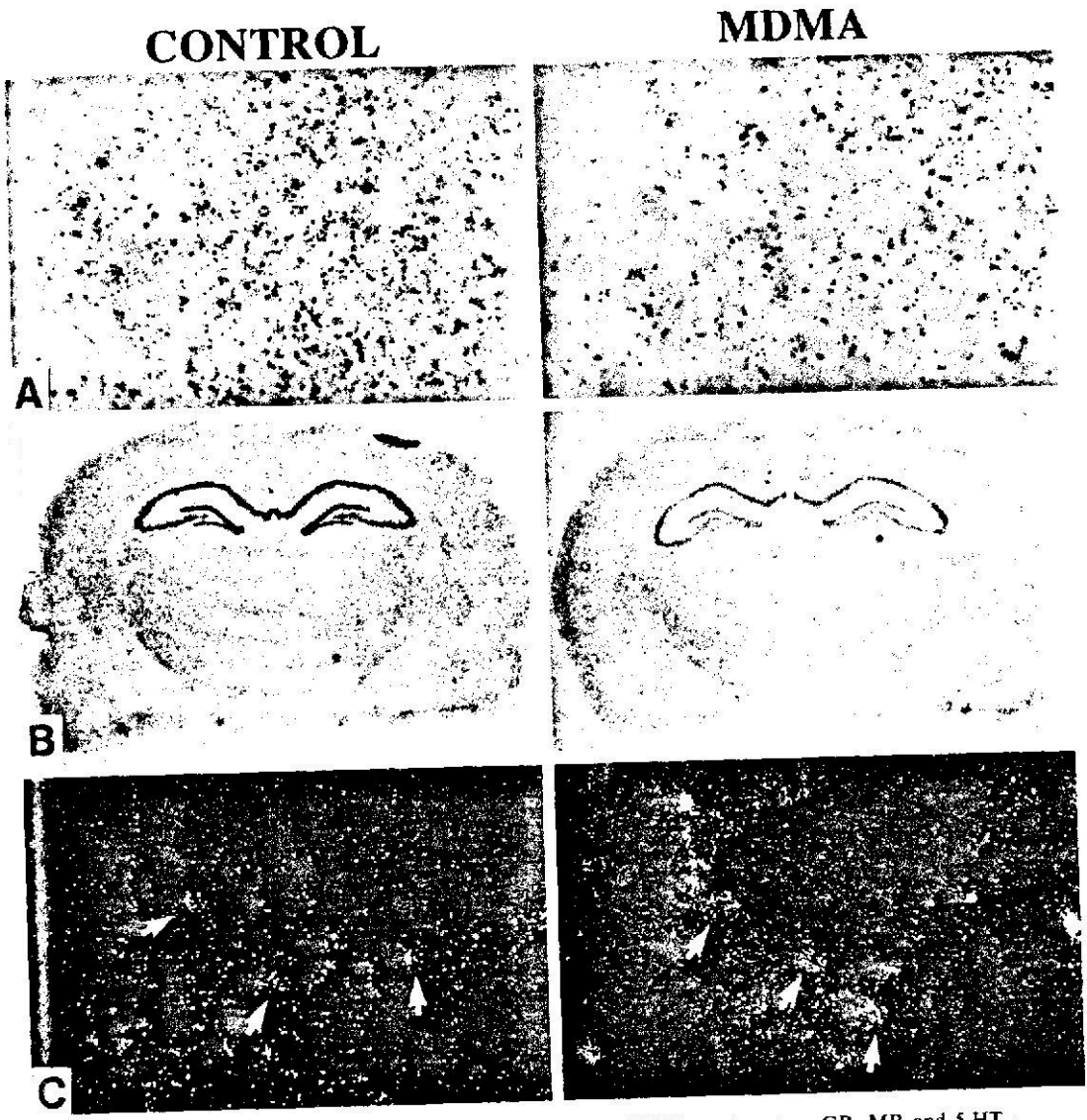

Fig. 2. Photomicrographs showing the effects of chronic MDMA treatment on GR, MR and 5-HT_{1C} receptor mRNA expression in the dorsal hippocampus. (A) Brightfield photomicrograph (× 428) of *in situ* hybridization showing GR mRNA expression in granule cells of the dentate gyrus in control and MDMA-treated rats. Autoradiographic silver grains appear black. Note attenuated expression following MDMA treatment. (B) Autoradiograph showing coronal brain sections from control and MDMA treated animals hybridised with antisense cRNA MR probe. Note attenuated hybridization signal in all hippocampal subregions following MDMA treatment. (C) Darkfield photomicrograph (× 85) of *in situ* hybridization showing 5-HT_{1C} receptor mRNA expression in CA3 cells of Ammon's horn. Autoradiographic silver grains appear white. Note increased expression per neuron (see arrow) following MDMA treatment.

Are changes at the level of gene expression reflected in altered corticosteroid binding sites? Recently, a single 20 mg/kg dose of MDMA was found to decrease GR binding sites in the rat striatum but not in the hippocampus, in contrast to our GR mRNA data.[30] Since both the dose and number of injections of MDMA affect the degree of neurotoxicity to 5-HT axons and terminals,[7] a single injection of MDMA may not be sufficient to affect 5-HT projections to the hippocampus and thus alter GR binding. In addition, Lowy *et al.*[30] did not adrenalectomize the rats prior to the binding assay to remove endogenous steroids and hence the results may merely reflect altered receptor occupancy. In a subsequent study in adrenalectomized rats, multiple injections of a related substituted amphetamine, methylenedioxyamphetamine, which also exerts toxic effects on 5-HT neurons, decreased GR binding in the hippocampus,[31] in agreement with our gene expression results.

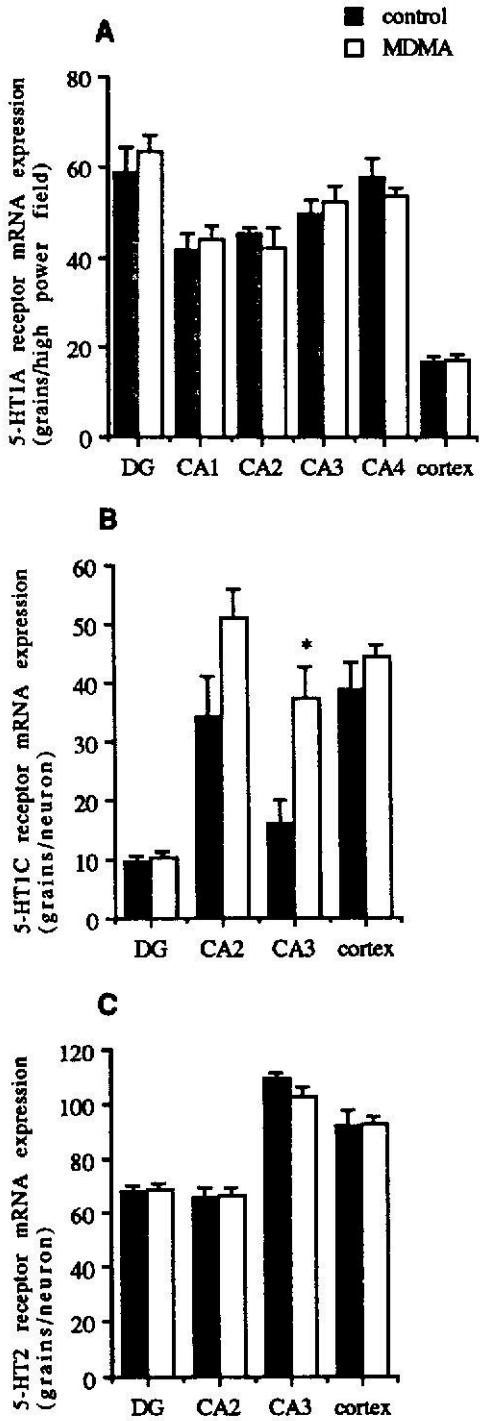

Fig. 3. Effect of chronic MDMA treatment on (A) 5-HT_{1A} (B) 5-HT_{1C} and (C) 5-HT_2 receptor mRNA expression in hippocampal subregions and cortex. Sprague–Dawley rats were given 0.9% saline or MDMA (20 mg/kg, s.c. twice daily for four days). Rats were killed 14 days after the last injection. Following hybridization of brain sections with the appropriate 5-HT receptor riboprobe as described in Experimental Procedures, slides were dipped in photographic emulsion, exposed for three to four weeks then developed and counterstained with haemotoxylin and eosin. DG, dentate gyrus. Results represent the mean ± S.E.M. ($n = 6$/group). *$P < 0.01$ compared with control.

The mechanisms whereby MDMA decreases hippocampal MR and GR mRNA expression is not known but is likely to relate to its neurotoxic actions on serotonergic nerve terminals and subsequent depletion of 5-HT. Thus, 5-HT administration to primary cultures of fetal hippocampal neurons leads to elevated GR binding sites.[39] *In vitro*, this effect is mediated via a ketanserin-sensitive pathway and involves the generation of intracellular cAMP[39]—rather unusually for presumed 5-$HT_{2/1C}$ receptor-mediated events which usually act via the phosphatidyl inositol pathway. The further intracellular events are obscure, but these data suggest that more indirect central or peripheral effects do not need to be invoked to explain the *in vivo* data presented here. Moreover, although we found elevated basal corticosterone levels after MDMA, expression of the mRNA species encoding GR and, more particularly, MR in the hippocampus show little long-term regulation by even gross manipulations of adrenal steroid levels.[13,24,49] Thus, Herman *et al.* [24] reported that large pharmacological doses of dexamethasone for eight days led to only a small reduction in GR mRNA expression restricted to CA3 with no changes in MR mRNA expression in any hippocampal subregion. These data do not suggest that effects of MDMA on circulating glucocorticoid levels themselves cause altered corticosteroid receptor gene expression in the hippocampus.

It seems more likely that elevated basal corticosterone levels reflect attenuated corticosteroid receptor expression in the hippocampus (and possibly other regions of the brain), since similar basal hypersecretion of glucocorticoids is seen in animals with reduced hippocampal corticosteroid receptor expression (e.g. in Brattleboro rats[54] or as a result of a stress-free neonatal environment[34]). Moreover, reversal of attenuated hippocampal GR expression (e.g. by neonatal handling or vasopressin replacement in Brattleboro rats) also reverses the circulating glucocorticoid abnormalities. The elevation of basal glucocorticoids as a result of MDMA treatment was clearly not sufficient (in magnitude or duration) to alter target organ (thymus) weight or target gene expression (CBG). In addition, adrenal weight was unaffected by MDMA treatment indicating no chronic elevation of ACTH. Recently adrenal hypertrophy was observed in about 30% of depressed patients,[42] indicating that chronic defective central control (possibly 5-HT-mediated) of the hypothalamic-pituitary-adrenal axis may associate with more than hypercortisolism. Although there have been reports of psychosis and depression induced by long-term MDMA abuse in humans[8,56] it is difficult to relate these cases directly to MDMA itself, and the mechanism underlying basal hypersecretion of corticosterone levels following chronic MDMA requires further investigation.

3,4-Methylenedioxymethamphetamine effects on 5-hydroxytryptamine receptor subtype mRNA expression

The rat hippocampus expresses a burgeoning variety of 5-HT receptors,[62] but most investigation has centred on 5-HT_{1A} and 5-$HT_{2/1C}$ subtypes. The particular 5-HT receptor subtype(s) involved in mediating 5-HT regulation of hippocampal MR and GR expression is not known, although *in vitro* data suggest a ketanserin-sensitive site.[39] Chronic MDMA treatment did not affect the expression of 5-HT_{1A} receptor mRNA expression in any hippocampal subregion. Under the same protocol, [^{3}H]80HDPAT binding to 5-HT_{1A} receptors in the hippocampus was also unchanged (Kelly, unpublished observations). A functional upregulation of 5-HT_{1A} receptors due to changes in the signal transduction processes beyond the receptors following MDMA treatment, however, may not be ruled out. Thus, a recent report showed functional up-regulation of 5-HT_{1A} receptors, as reflected in behavioural data, in the absence of changes to receptor binding site following chronic antidepressant treatment.[32]

In agreement with our results, the selective lesioning of serotonergic neurons by intra-raphe administration of 5,7-DHT had no effect on 5-HT_{1A} receptor mRNA expression in the hippocampus[38] and extensive degeneration of serotonergic projections did not alter 5-HT_{1A} binding sites on post-synaptic neurons.[23,61] In contrast, Brousseau *et al.*,[10] found increased 5-HT_{1A} receptor mRNA expression in the rat hippocampus 11 days following the injection of 5,7-DHT. Whereas 5,7-DHT produces a marked and relatively uniform decrease in 5-HT uptake sites (84–90% in hippocampal subregions),[23] MDMA treatment causes an equally marked, but regionally more heterogeneous reduction of 5-HT uptake sites, which in this study ranged from 44% in dentate gyrus to 92% in CA2 (cf. 60–80%).[6] The differing effects of 5,7-DHT, MDMA and various experimental paradigms on 5-HT_{1A} sites may thus reflect differences in the completeness of lesions innervating particular hippocampal subfields.

Chalmers *et al.*[12] recently reported increased 5-HT_{1A} receptor mRNA expression in all hippocampal subfields one week following adrenalectomy, an effect which was preventable by administration of exogenous corticosterone at the time of adrenalectomy. In contrast, we find no adrenal steroid regulation of hippocampal 5-HT_{1A} receptor mRNA expression following MDMA treatment in spite of increased basal corticosterone levels. Other studies have shown 5-HT_{1A} receptor mRNA expression in the hippocampus to be unaltered[25a] in all subfields and increased[34a] or decreased[28a] only in the dentate gyrus following adrenalectomy. The different experimental protocols and status of the rats, however, make it difficult to compare these studies directly to ours; for example the serotonergic innervation to the hippocampus in the adrenalectomized rats remain intact in contrast to our MDMA treated rats.

Chronic MDMA treatment also had no effect on the expression of 5-HT_2 receptor mRNA in any hippocampal subregion. In agreement with our gene expression data [^{3}H]ketanserin binding to 5-$HT_{2/1C}$ receptors in hippocampal homogenates were unaltered under the same MDMA protocol (Kelly, unpublished observations). Other reports of serotonergic denervation have been inconclusive, showing a lack of change[5,18] decreased[29,55] or increased 5-HT_2 receptor density.[21] Some of these discrepancies reflect differences in the acute and chronic actions of MDMA; the transient down-regulation of 5-HT_2 receptors 6 h after MDMA is most probably due to the acute discharge of 5-HT into the synaptic cleft.[55]

By contrast, we found that chronic MDMA administration significantly increased 5-HT_{1C} receptor mRNA expression in CA3, although not in CA2 or other regions expressing this gene. The increased expression of 5-HT_{1C} receptor mRNA is likely to reflect a direct stimulus to up-regulate synthesis (and expression) of this postsynaptic receptor following 5-HT denervation by chronic MDMA treatment, since glucocorticoid manipulations (removal of endogenous steroids by adrenalectomy and steroid replacement by either aldosterone or dexamethasone) *per se* do not alter 5-HT_{1C} receptor mRNA expression in the dorsal hippocampus.[25a] The subtle change in 5-HT_{1C} receptor gene expression in a specific subregion of the hippocampus may not be detected as an increase in 5-HT_{1C} receptor binding since (i) in radioligand binding studies whole hippocampus is used and (ii) there is as yet no specific radioligand which can distinguish between 5-HT_{1C} and 5-HT_2 receptors; the greater abundance of 5-HT_2 receptor sites may mask small changes in 5-HT_{1C} receptor sites. The lack of change in the binding of the 5-$HT_{1C/2}$ receptor radioligand [^{125}I]MIL) in the hippocampus one week after chronic MDMA treatment[55] is thus not necessarily incompatible with our finding of increased 5-HT_{1C} receptor gene expression. Since 5-HT_{1C} receptor mRNA expression and binding sites are not found in all hippocampal subfields, whereas MDMA decreased MR and GR mRNA expression in all hippocampal subregions, the involvement of other 5-HT receptor subtypes not examined in the present study may not be ruled out, although these effects may be remotely mediated via the intrahippocampal circuitry. It is conceivable that the 5-HT_{1C} receptor mediates the effects of ketanserin and other 5-$HT_{1C/2}$ receptor antagonists[20] on MDMA-mediated processes, including corticosterone hypersecretion.[41] Similarly, these agents antagonize the effects of 5-HT to increase GR binding sites in primary hippocampal neuronal cultures, actions thought to relate to the 5-HT_2 receptor.[39] However, our and other data suggest that the 5-HT_{1C} subtype may be important in mediating some of the hippocampal actions of 5-HT.

Thus, 5-HT_{1C} receptors are prominent in the neonatal hippocampus where they link to second messenger pathways.[14] Furthermore, supersensitivity of 5-HT_{1C} receptors is seen after 5,7-DHT lesions,[53] suggesting that increased expression of this receptor subtype occurs with other 5-HT lesions.

CONCLUSIONS

The data presented here demonstrate that chronic treatment with MDMA results in a marked decrease in expression of GR and MR mRNAs in all hippocampal subregions and increased 5-HT_{1C} receptor mRNA in CA3 neurons. Whether the decreased expression of MR and GR mRNA is associated with corresponding decreased receptor levels remains to be determined although this seems likely since another method of producing 5-HT depletion using 5,7-DHT resulted in both decreased hippocampal MR and GR mRNA expression and corticosteroid receptor binding sites.[57,60] To determine whether the increased expression of 5-HT_{1C} receptor mRNA reflects an increase in binding sites awaits the availability of a highly selective ligand which does not bind to 5-HT_2 receptors. The long-term neural consequences of MDMA abuse in man is not known. However, the selective vulnerability of rat hippocampal corticosteroid receptor mRNAs to chronic MDMA exposure, taken together with data indicating that primates have similar 5-HT neuronal sensitivity to MDMA and even less reversability of neurotoxicity than rodents,[51,52] suggests that MDMA abuse may also lead to long term neuroendocrine and affective dysfunction in humans.

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