ORIGINAL ARTICLE

NEUROCHEMICAL AND BEHAVIORAL EFFECTS OF M-CPP IN A RAT MODEL OF TARDIVE DYSKINESIA

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ABSTRACT

Present study was designed to monitor the responsiveness of 5HT (5-Hydroxytryptamine) -2C receptors following the long-term administration of haloperidol in rats. Effects of m-CPP (meta-Chlorophenyl piperazine) were monitored 48h after withdrawal from repeated (twice a day for 5 week) administration of haloperidol (at the dose of 1mg/kg). Vacuous chewing movements (VCMs) were monitored on weekly basis. Two days after withdrawal, animals were injected with saline (1ml/kg of body weight) or m-CPP (3mg/kg of body weight). Activities in open field and light dark activity box were monitored 15 and 30min post injection respectively.

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Animals were then decapitated (4h post injection) to collect dorsal striatum (DS) samples for the neurochemical analysis by HPLC-EC (High Performance Liquid Chromatography with Electrochemical detection) method. Results from the present study showed significant hypolocomotive effect of m-CPP ($p<0.05$) in both repeated haloperidol as well as repeated saline injected rats. Neurochemical analysis of DS by HPLC-EC method showed that administration of m-CPP significantly ($p<0.05$) decreased 5-HIAA (5-Hydroxyindol acetic acid) in repeated haloperidol injected rats. In conclusion, present study provides evidence that 5HT-2C receptors become hypersensitive in a rat model of Tardive Dyskinesia (TD). These findings have potential implication in the treatment of TD and attenuation of EPS induced by typical neuroleptics.

Keywords: Haloperidol, extra pyramidal side effects, tardive dyskinesia, m-CPP, 5HT-2C receptors.

INTRODUCTION

Schizophrenia is a thought disorder and is characterized both by disturbed form and content of thoughts (Cohen *et al*., 2006). Typical antipsychotics such as haloperidol and chlorpromazine are widely prescribed for the treatment of schizophrenia but their beneficial side effects are accompanied by extra pyramidal side effects (EPS) (Yamada *et al*., 2002). The short term effects include Parkinsonism (Glazer, 2000), whereas tardive dyskinesia appear much later (Klawans, 1985), which could be due to the hypersensitivity of (Dopamine-2) D2 receptors which develops upon prolonged haloperidol administration (Kulkarni and Naidu, 2000).

In addition to dopamine receptors, serotonin receptors are also important in the etiology of schizophrenia and in the elicitation of EPS (Castensson *et al*., 2005). Multiple receptors for 5-Hydroxytryptamine (5HT) exist in the central nervous system, 5HT-2C receptors are present on the cell body and dendrites of dopaminergic neurons and negatively regulate the release of dopamine (Esposito, 2006).

m-CPP, a metabolite of trazodone (Rotzinger *et al*., 1998) is an agonist to both 5HT-1B and 5HT-2C receptors (Gommans *et al*., 1998). Acting via 5HT-2C receptors, it decreases the release of dopamine (Di Giovanni *et al*., 2000) which could be attenuated by selective 5HT-2C receptor antagonists (Di Matteo *et al*., 1999). While acting via 5HT-1B receptors, it regulates the release of 5HT (Eriksson *et al*., 1999).

5HT-2C receptors may be involved in the pathophysiology of hyperkinetic movement disorders (Mehta *et al*., 2001). Neurochemical research showed that a decrease in striatal serotonin metabolism following the administration of clozapine is involved in its beneficial therapeutic profile and ability to produce fewer EPS than haloperidol (Haleem *et al*., 2002a).

The purpose of present study was to monitor the responsiveness of 5HT-2C receptors following the long-term administration of haloperidol in rats.

METHODS AND MATERIALS

Animals

Twenty-four female Albino Wistar rats, weighing 180-200g were purchased from HEJ Research Institute of Chemistry, Karachi, Pakistan. The animals were housed individually in plastic cages under a 12h light-dark cycle (lights on at 6:00 h) with free access to tap water and cubes of standard rodent diet for at least one week before the start of experiment, so that, they could become familiar to the environment. They were accustomed to various handling procedures to nullify stress effects. All experiments were performed according to the protocols approved by the local animal care committee.

Drugs

Haloperidol (Serenace, Searle, USA), purchased as injectable ampoules of 5mg/ml, was injected intraperitoneally at a dose of 1mg/kg body weight. m-CPP 2HCl, purchased from Sigma (Haleem, 1993) was dissolved in saline and injected intraperitoneally at the dose of 3mg / ml / kg body weight. Control animals were injected with saline in volumes of 1ml/Kg body weight.

Experimental protocol

Twenty-four female rats were randomly divided into four groups, each containing six animals: (i) Repeated saline plus saline, (ii) Repeated saline plus m-CPP, (iii) Repeated haloperidol plus saline, (iv) Repeated haloperidol plus m-CPP injected rats. These four groups of animals were then injected repeatedly (twice a day at 9:00 – 9:30 and 13:30 – 14:00 for 5 weeks) with saline (1ml/kg of body weight) or haloperidol (1mg/kg of body weight). Vacuous chewing movements were recorded on weekly basis. Two days after withdrawal; animals were injected with saline (1ml/kg of body weight) or m-CPP (3mg/ml/kg of body weight). Activities in open field and light-dark activity box were monitored 15 and 30 min post injection respectively. The animals were then decapitated 4h after the acute administration of m-CPP or saline to collect dorsal striatum samples for the neurochemical analysis.

Monitoring vacuous chewing movements, open field and light-dark activities

Activity in open field was monitored 15 min post

injection. The procedure was essentially similar as described before (Haleem *et al*., 2002c). Animals were placed in the central square of the open field and numbers of squares crossed with all four paws were counted for 5 minutes. Vacuous chewing movements were recorded in the home cage apparatus as described elsewhere (Goel *et al*., 2005). Each burst of purposeless chewing movements, which remained continuous for at least 30sec, was counted as one. Light dark activity was monitored 30min post injection and number of entries in the light compartment was recorded (Langen *et al*., 2005).

Dissection of striatum

Dissection procedure was essentially same as described elsewhere (Haleem *et al*., 2004). After decapitation, fresh brain was dipped in ice-cold saline and placed with its ventral side up in the molded cavity of brain slicer. Fine razor/blade was inserted between the slots of slicer to give slice of 2mm thickness. The slice containing striatum was transferred to a slide kept on ice. Punches of 2.5mm diameter were made bilaterally in the striatum to collect dorsal striatum (DS). Samples were frozen at -70ºC until analysis by HPLC-EC.

HPLC-EC determination of 5-HT, DA and their metabolites

HPLC-EC determination was carried out as described before (Haleem *et al*., 2002b; Haleem *et al*., 2004). A 5µ Shim-pack ODS separation column of 4.0 mm internal diameter and 150mm length was used. Separation was achieved by a mobile phase containing methanol (14%), octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1 M phosphate buffer of PH 2.9 at an operating potential of 2000-3000 psi on Schimadzu HPLC pump. Electrochemical detection was achieved on Schimadzu LEC 6A detector at an operating potential of +0.8V.

STATISTICAL ANALYSIS

Results are represented as means ± S.D. Statistical analysis was performed by two-way analysis of variance (ANOVA), to see the effect of various factors involved. Post hoc comparison of groups was performed by Newman-Keuls test. Values of $p < 0.05$ and < 0.01 were considered as significant.

RESULTS

Tardive dyskinesia induced by repeated haloperidol administration

Fig.1 shows effect of repeated administration of haloperidol on vacuous chewing movements (VCMs). Two-way ANOVA revealed significant effect of both haloperidol administration ($F=928.8$, $df=1,132$, $p<0.01$) as well as weekly monitoring (($F=42.0$, $df=4,132$, $p<0.01$). Interaction between the two factors was found to be significant (($F=218.34$, $df=4,132$, $p<0.01$). Post hoc analysis by Newman-Keuls test revealed significant ($p<0.01$) increase in the vacuous chewing movements by haloperidol administration after 1st week, which persisted till 5th week of drug administration. The effect after 1st injection was not significant.

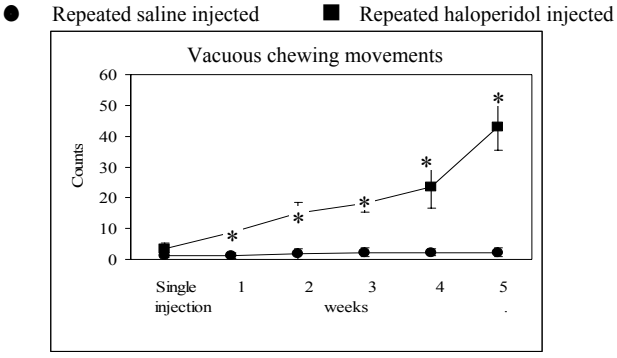

Fig. 1: Effect of repeated administration (two times a day for 5 weeks) of haloperidol (1mg/kg) on vacuous chewing movements in rats. Values are means ±S.D. (n=12). Significant differences by Newman-Keuls test: *P<0.01 in repeated haloperidol injected rats from their respective repeatedly saline injected controls following two-way ANOVA.

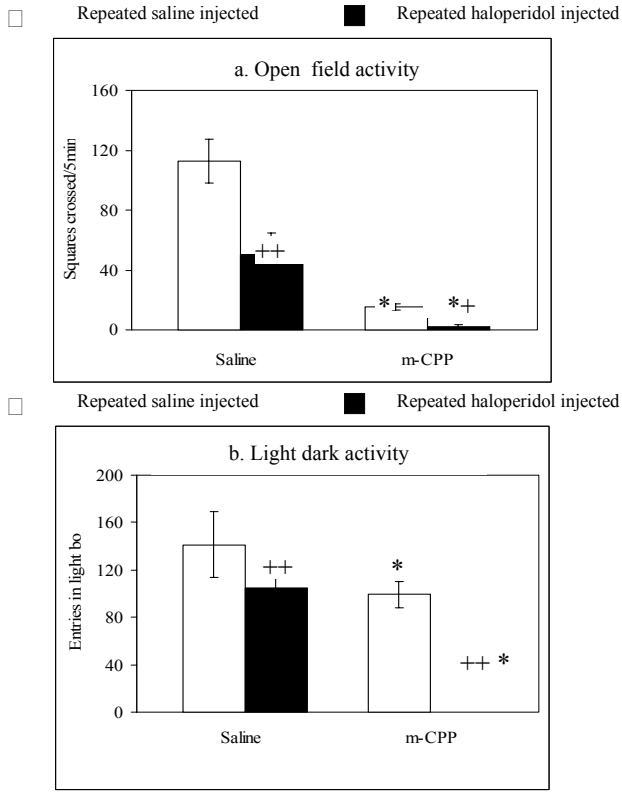

Fig. 2: Effect of single dose of m-CPP (3mg/kg) in rats repeatedly (two times a day for 5 weeks) injected with haloperidol (1mg/kg) or saline (1ml/kg), on open field and light-dark activities. Values are means ±S.D. (n=6). Significant differences by Newman-Keuls test: *p<0.01 from saline injected controls; +p<0.05, ++p<0.01 from repeated saline injected controls following two-way ANOVA.

Hypolocomotive and anxiogenic effects of m-CPP in a rat model of TD

Fig. 2(a) shows the effect of single dose of m-CPP in rats following withdrawal from repeated administration of haloperidol on open field activity. Two-way ANOVA (df=1, 20) revealed significant effect on open field activity, of haloperidol (F=80.2, p<0.01) as well as m-CPP (F=292.6, p<0.01) administration. The interaction between the two factors was also significant (F=34.21, p<0.01). Post hoc analysis by Newman-Keuls test showed that m-CPP significantly decreased number of squares crossed in both repeated saline- (p<0.01) as well as repeated haloperidol-injected rats (p<0.01) from their respective repeated saline plus saline and repeated haloperidol plus saline injected rats. Repeated haloperidol plus saline- (p<0.01) and repeated haloperidol plus m-CPP-injected (p<0.01) rats showed decrease in the number of squares crossed in the open field from their respective repeated saline plus saline and repeated saline plus m-CPP injected controls.

Fig. 2(b) shows the effect of single dose of m-CPP in rats following withdrawal from repeated administration of haloperidol on light-dark activity. Two-way ANOVA (df=1, 20) revealed significant effect on light-dark activity, of both haloperidol (F=86.0, p<0.01) as well as m-CPP (F=101.5, p<0.01) administration. The interaction between the two was also found to be significant (F=18.15, p<0.01). Post hoc analysis by Newman-Keuls test showed that m-CPP significantly decreased number of entries in the light box in both repeated saline-(p<0.01) as well as repeated haloperidol-injected rats (p<0.01) from their respective repeated saline plus saline and repeated haloperidol plus saline injected rats. Values were smaller in both repeated haloperidol plus saline- (p<0.01) as well as repeated haloperidol plus m-CPP injected rats (p<0.01) from their respective repeated saline plus saline and repeated saline plus m-CPP injected controls.

Effect of m-CPP on 5-HT metabolism in dorsal striatum of a rat model of TD

Fig. 3 shows the effect of single dose of m-CPP on levels of 5-HT and 5-HIAA in the DS of rats following withdrawal from repeated haloperidol administration. Two-way ANOVA (df=1, 20) revealed that the effects of both haloperidol (F=2.12, p>0.05) and m-CPP administration (F=2.74, p>0.05) on 5-HT levels as well as interaction between the two (F=18.0, p>0.05) were not significant. While there was significant (F=0.308, p<0.01) effect of m-CPP administration on 5-HIAA levels. The effect of haloperidol (F=0.308, p>0.01) as well as interaction between these two (F=3.48, p>0.05) were not significant. Post hoc analysis by Newman-Keuls test showed significant (p<0.05) decrease by m-CPP in 5-HIAA levels of repeated haloperidol injected animals from their respective repeated haloperidol plus saline injected controls.

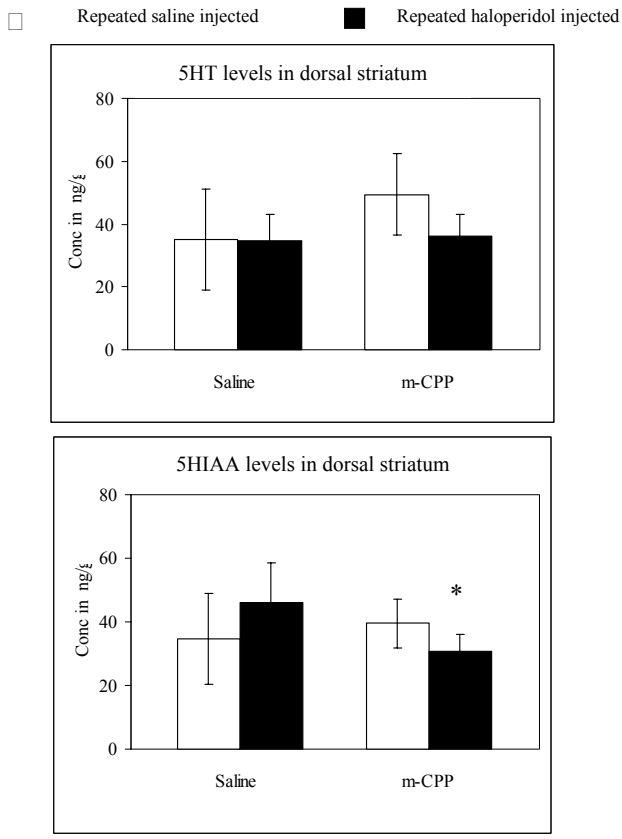

Fig. 3: Effects of single dose of m-CPP (1mg/ml/kg) on levels of 5-HT and 5-HIAA in the dorsal striatum of rats repeatedly (two times a day for 5 weeks) injected with saline (1ml/kg) or haloperidol (1mg/kg). Values are means ±S.D. (n=6).* p<0.05 from saline injected controls following two - way ANOVA.

Effect of m-CPP on dopamine metabolism in DS of a rat model of TD

Fig. 4 demonstrates the effect of single dose of m-CPP on levels of Dopamine (DA), Dihydroxy Phenyl Acetic Acid (DOPAC) and Homovalinic acid (HVA) in DS following withdrawal from repeated haloperidol administration. Two-way ANOVA (df=1, 20) revealed that the effect of haloperidol (F=1.36, p>0.05) and m-CPP administration (F=2.09, p>0.05) on DA levels as well as interaction between the two (F=0.149, p>0.05) were not significant. There was a significant effect of m-CPP administration (F=14.59, p<0.01) on DOPAC levels and the interaction between the two (F=9.57, p<0.01) was also significant but the effect of haloperidol on DOPAC levels was not significant (F=1.21, p<0.01). Effect of m-CPP (F=22.98, p<0.01) and haloperidol administration (F=16.76, p<0.01) on HVA levels as well as interaction between the two (F=5.55, p<0.05) were all significant. Post hoc analysis by Newman-Keuls test showed that haloperidol significantly (p<0.01) increased HVA levels in repeated haloperidol injected rats.

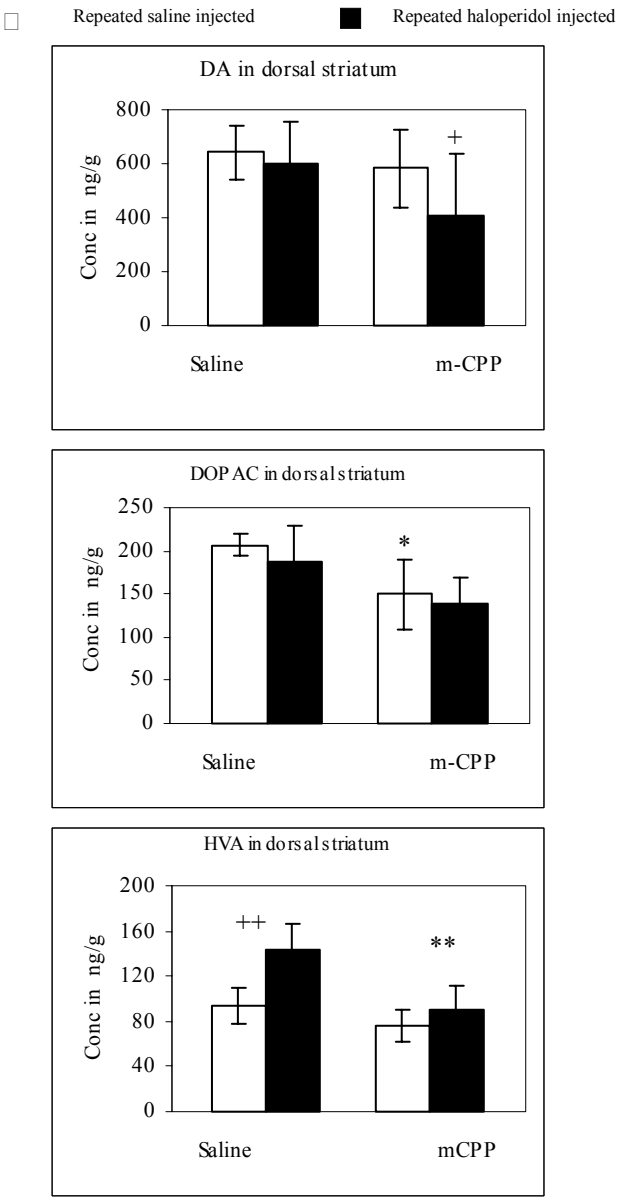

Fig. 4: Effect of single dose of m-CPP (3mg/ml/ kg) on the levels of dopamine, DOPAC and HVA in the dorsal striatum of rats repeatedly (two times a day for 5 weeks) injected with saline (1ml/kg) or halopeidol (1mg/kg). Values are means ±S.D.(n=6). Significant differences by Newman-Keuls test: +p<0.05, ++ p<0.01 from repeated saline injected injected controls; *p<0.05, **P<0.01 from saline injected controls following two-way ANOVA.

DISCUSSION

Tardive dyskinesia induced upon repeated haloperidol administration

In the present study, rat model of tardive dyskinesia (Ikeda *et al*., 1999) was used to monitor responsiveness of 5HT-2C receptors (Fig. 1). Tardive dyskinesia appears after the long term treatment of haloperidol and D2 receptor hypersensitivity as a result of chronic treatment with haloperidol (Kulkarni and Niadu, 2000) might be the cause of it. The repeated jaw movement responses remain high after drug washout and haloperidol during this period was found to contain 70% D2 receptor occupancy (Rosengarten and Quartermain, 2003).

Hypolocomotive effects of m-CPP in repeated haloperidol injected rats

Typical effect of administration of a D2 receptor antagonist such as haloperidol is suppression of spontaneous locomotor and exploratory activity (Haleem *et al*., 2004) in a dose dependant manner (Alamy *et al*., 2005). Basic reason of this markedly decreased locomotor and exploratory activity could be increased anxiety level, as suggested by Karl *et al*., (2006). A study conducted by Singh *et al*., (2002) also demonstrated that offspring of rats treated with haloperidol (2.5 mg/kg) from gestation day 12 to 21 (which is the critical period for neural development) showed diminished locomotor activities in an open field as well as increased anxiety levels. Other scientists have also reported hypolocomotory effects induced by haloperidol administration, because of D2 receptor blockade as well. (Ali *et al*., 2005; Bardin *et al*., 2005).

In the present study, m-CPP significantly decreased locomotor activity in both repeated haloperidol as well as repeated saline injected rats (Fig. 1). This could be due to increased anxiety levels because of binding of m-CPP with 5HT-2C receptors (Gatch, 2003). These hypolocomotive responses by m-CPP increase with high doses of m-CPP (Haleem, 1993). Local infusion of m-CPP into different brain regions has shown to reduce exploratory activity as well as locomotor activity. These responses were attenuated by 5HT-2C receptor antagonist SB-242084, which confirms that m-CPP produces decreased locomotor activity via 5HT-2C receptors (Campbell and Merchant, 2003). The stimulation of 5HT-2C receptors by selective 5HT2C receptor agonists decreases the release of dopamine, while the antagonists of these receptors enhance the dopaminergic function (Esposito, 2006). m-CPP being an agonist to 5HT2C receptors, decreases the release of dopamine (especially in mesolimbic area) induced by selective antagonists of these receptors (Alex *et al*., 2005). This decreased release of dopamine could account for hypolocomotion as decreased dopaminergic function leads to hypolocomotory effects, since dopamine is involved in the regulation of locomotion. Tolerance develops to the hypolocomotive effects of m-CPP upon repeated administration.

Anxiogenic effects of m-CPP in repeated haloperidol injected rats

Haloperidol upon administration produces impaired working memory and increased anxiety levels (Karl *et al*.,

2006). Serotonin may be involved in this haloperidol induced increased anxiety levels, since 5-HT levels increase upon the administration of haloperidol (Haleem *et al*., 2002a). The present study showed marked increase in anxiety levels following acute administration of m-CPP in rats pre-treated with haloperidol or saline (Fig. 2b). m-CPP has also been reported to diminish exploratory activity of animals in the light compartment of light-dark apparatus at a dose of 0.5 mg/kg (Bilkei *et al*., 1998). m-CPP is a mixed agonist at 5HT-1B and 5HT-2C receptors but m-CPP-induced anxiety is mediated by post-synaptic 5HT-2C receptors (Alves *et al*., 2004). These anxiogenic responses were more pronounced in repeated haloperidol injected animals (fig. 2a). This could be explained on the basis of 5HT-2C receptor hypersensitivity upon prolonged treatment with haloperidol.

Effect of m-CPP on 5-HT metabolism in DS of repeated haloperidol injected rats

In the present study, administration of haloperidol did not altered 5-HT levels in the dorsal striatum (fig. 3). Increase in 5-HT levels in the striatum, upon haloperidol administration had been reported in other papers from our laboratory (Ali *et al*., 2005; Haleem *et al*., 2002). In the present study, administration of m-CPP decreased 5-HIAA levels in the dorsal striatum of rats repeatedly injected with haloperidol (fig. 3b). This could be explained on the basis of affinity of m-CPP for 5HT-1B receptors. These 5HT-1B receptors are present presynaptically on the terminals of striatal serotonergic neurons and regulate the release of 5-HT (Maroteaux *et al*., 1992).

Effect of m-CPP on DA metabolism in DS of repeated haloperidol injected rats

The typical response to first generation neuroleptics such as haloperidol, is associated with an increase in HVA concentration. The proposed mechanism of HVA changes involves blockade of both pre- and post-synaptic dopamine D2 receptors (Haleem *et al*, 2004). The presynaptic blockade increases the amount of dopamine released by a subsequent action potential and because dopamine D2 postsynaptic receptors are also blocked, acute dopamine neurotransmission is attenuated. The effect of haloperidol on increased HVA levels in the dorsal striatum (fig. 4c) is consistent with previous reports of our laboratory which demonstrated similar results in whole striatum (Shireen *et al*., 2002). The increased HVA is the predictor of response to neuroleptics (Friedhoff and Silva, 1995). The decrease in DOPAC and HVA levels upon administration of m-CPP, in the dorsal striatum of repeated haloperidol injected rats (Fig 4b & 4c) is also explainable in the terms of binding of m-CPP with 5HT-2C receptors present pre-synaptically on dopaminergic neurons (Lucas *et al*., 2000) resulting in the decreased dopamine release as well as its degradation to HVA. Stimulation of 5HT-2C receptors by systemic administration of 5HT-2C agonist has been shown to increase dopamine metabolism in the prefrontal cortex (Westerink *et al*., 2001). Thus decreased release of dopamine as a result of 5HT-2C receptor stimulation, is possibly involved in hypolocomotory effects of m-CPP (fig 3a). Acute hypolocomotory effects of m-CPP upon acute administration, may result because of decreased release of dopamine due to simulation of 5HT-2C receptors.

It was observed from present study that the decrease of dopamine release upon m-CPP administration was more pronounced in repeated haloperidol administered animals, which could be due to the up-regulation of 5HT-2C receptors following long-term administration of haloperidol.

CONCLUSION

An increase in the responsiveness of m-CPP as observed in the present study, could be explained in terms of increased effectiveness of 5HT-2C receptors in the rat model of tardive dyskinesia present presynaptically on dopaminergic neurons. In conclusion, present study provides evidence that 5HT-2C receptors are supersensitized by long-term administration of haloperidol. It would suggest that drugs with antagonistic activity for 5HT-2C receptors may prove useful in the suppression of tardive dyskinesia in patients treated with the typical neuroleptics.

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