Methcathinone is a Substrate for the Serotonin Uptake Transporter*

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Abstract: We previously reported that the psychostimulant drug methcathinone inhibits serotonin accumulation via the plasma membrane serotonin uptake transporter. By analogy to known substrates for the serotonin transporter, we hypothesized that methcathinone is also a substrate for this transporter and that inhibition of serotonin uptake by methcathinone occurs in part through competition for substrate recognition sites within the transporter. To test the hypothesis we preloaded human platelets with \[^3H\]5-HT then superfused the platelets with either methcathinone or with the known serotonin uptake transporter substrate para-methoxyamphetamine. Under superfusion conditions, transporter substrates will evoke an increase in released \[^3H\]5-HT through a carrier-mediated exchange process. For direct assessment of methcathinone transport via the serotonin uptake transporter, we tested whether \[^3H\]methcathinone would be accumulated by cells stably expressing the cloned human serotonin uptake transporter (293SERT cells). Supporting the hypothesis, superfusion of \[^3H\]5-HT-containing platelets with methcathinone or with para-methylthioamphetamine produced a large increase in tritium efflux. The efflux declined when the drugs were removed. When increasing concentrations of \[^3H\]methcathinone were incubated with 293SERT cells under conditions used to assess serotonin transport, saturable, single-site accumulation of radiolabel was observed. The uptake of \[^3H\]methcathinone was temperature, inhibitor, and sodium-sensitive, and was not observed in wild-type HEK 293 cells. Non-linear regression analysis of specific \[^3H\]methcathinone uptake produced values for \(K_{d}\) and \(V_{max}\) of 244±51 nM and 202±25 fmol/min/mg protein, respectively. These data support the notion that the reported serotonergic neurotoxicity of methcathinone may arise through accumulation of the drug within serotonergic neurones.

Methcathinone (N-methylcathinone; 2-methylamino-1-phenylpropan-1-one) is a potent psychostimulant drug that resembles cocaine or amphetamine in its behavioural effects. Researchers have reported that methcathinone is self-administered by baboons (Kaminski & Griffiths 1994) and that methcathinone will substitute for either amphetamine or cocaine in animals trained to discriminate these drugs from vehicle (Glennon et al. 1995; Young & Glennon 1993 & 1998).

Methcathinone affects several neurochemical parameters in the rat brain. Methcathinone causes the release of dopamine from brain tissue preloaded with \[^3H\]dopamine (Glennon et al. 1987) or under conditions of microdialysis (Gygi et al. 1997), suggesting that the drug is a dopamine transporter substrate. The activities of two neurotransmitter biosynthetic enzymes, tyrosine hydroxylase and tryptophan hydroxylase, are decreased following methcathinone administration, leading to reductions in the concentrations of dopamine and serotonin and their respective metabolites in frontal cortex, hippocampus, and neostriatum (Gygi et al. 1996). The drug also produces a decrease in dopamine and serotonin uptake transporter function (Sparago et al. 1996; Gygi et al. 1997; Metzger et al. 1998; Fleckenstein et al. 1999). It is believed that these deficits, similar to those seen with neurotoxic phenylalkylamines (Ricaurte et al. 1985; Schuster et al. 1986; Molliver et al. 1990), may reflect potential long-term damage to dopaminergic and serotonergic neurones. Nevertheless, to become evident, these neural deficits require massive, multiple doses of methcathinone (e.g. 4×30 mg/kg over 12 hr, or 8×50 mg/kg over 4 days). Such doses are 10 to 100 times higher than behaviourally active doses (Glennon et al. 1987; Gygi et al. 1996; Sparago et al. 1996; Metzger et al. 1998). Deficits in dopamine function, but not serotonin function, were prevented by pretreatment with dopamine D1 or D2 receptor antagonists. However, serotonergic changes could be prevented if rats were depleted of striatal dopamine by lesioning with 6-hydroxydopamine (Gygi et al. 1997). Apparently the serotonergic neurotoxicity of methcathinone is promoted by the presence of the N-methyl group on the drug molecule because it was earlier reported that no long-term changes in serotonin levels were observed with repeated high doses of the desmethyl parent compound, cathinone (Wagner et al. 1982).

Little is known about the actions and effects of methcathinone in human tissues. In an earlier study, we reported that methcathinone inhibits \[^3H\]norepinephrine and \[^3H\]serotonin (\[^3H\]5-HT) accumulation via human monoamine uptake transporters expressed in transfected cells or platelets, respectively (Cozzi et al. 1999). Markantonis et al. (1986) examined the metabolism of methcathinone in man...
and Russian scientists have reported visceral and vascular pathologies in methcathinone users (Mamrova et al. 2001; Pigolkin Iu & Sherstiuk 1996). McCann et al. (1998) reported that humans with a history of methcathinone use exhibited decreased densities of dopamine uptake transporters as estimated by positron emission tomography, suggesting that methcathinone is toxic to dopamine neurones in human users.

If methcathinone-induced neurotoxicity relies on the same mechanism reported for other neurotoxic phenylalkylamines, then transport of methcathinone into the neurone is a necessary first step in the production of neurotoxicity. The evidence that methcathinone is a substrate for dopamine transporters in rats would seem to support this mechanism at dopaminergic neurones. One of the goals of the present study was to determine whether methcathinone was also a substrate for the serotonin transporter. We hypothesized that methcathinone is a substrate for the serotonin transporter and we tested this hypothesis and evaluated the kinetic parameters of methcathinone uptake via the serotonin transporter expressed in a heterologous cell line.

A serotonin transporter substrate will evoke the release of stored cytosolic serotonin through a transporter-mediated exchange mechanism (Rudnick 1997). The ability of a drug to elicit serotonin release is thus a diagnostic criterion for a serotonin transporter substrate. To test the hypothesis that methcathinone is a substrate for the serotonin transporter, we examined drug effects in human platelets and in cells transfected with the cloned human serotonin uptake transporter. We preloaded human platelets with \( ^{3} \text{H} \)-HT and superfused them with methcathinone as an initial screen to see if we could observe drug-evoked \( ^{3} \text{H} \)-HT release. We also applied the known serotonin releaser para-methoxyamphetamine (Huang et al. 1992; Gobbi et al. 2002) as a positive control. An increase in the amount of tritium released was taken as evidence that the superfused drug is a serotonin transporter substrate. For direct assessment of methcathinone transport via the serotonin uptake transporter and to determine \( K_M \) and \( V_{max} \) values, we tested whether \( ^{3} \text{H} \)-methcathinone would be accumulated by wild-type human embryonic kidney 293 (HEK 293) cells or by HEK 293 cells stably expressing the cloned human serotonin uptake transporter (293SERT cells) under a variety of conditions.

Materials and Methods

Drugs and reagents. Racemic methcathinone hydrochloride for \( ^{3} \text{H} \)-HT release experiments was synthesized from (+)-ephedrine as described by Zhengel et al. (1991). For direct assessment of methcathinone uptake, we synthesized racemic \( ^{3} \text{H} \)-methcathinone with a specific activity of 80 Ci/mmol in two steps from phenylpropanolamine as previously reported (Cozzi & Ruoho 1998). Briefly, phenylpropanolamine was oxidized to cathinone using potassium permanganate in acetic acid. The cathinone produced was then N-methylated with \( ^{3} \text{H} \)-methyl iodide in a mixture of toluene and methanol. The product was purified by reverse-phase HPLC to give \( ^{3} \text{H} \)-methcathinone (2,4-\( ^{3} \text{H} \)-methylenamino-1-phenylpropan-1-one). Racemic para-methoxyamphetamine hydrochloride was synthesized by Dr. Aaron Monte (University of Wisconsin-LaCrosse). Physical and chemical analytical data for all the synthesized compounds were consistent with the expected structures. \( ^{3} \text{H} \)-HT (specific activity=27.5 Ci/mmol) was purchased from New England Nuclear (Boston, MA, USA). Cell culture medium and antibiotics were obtained from Life Technologies (Gaithersburg, MD, USA). Foetal bovine serum was purchased from Hyclone (Logan, UT, USA). Paraglyne, buffer salts, and miscellaneous chemicals were acquired from Aldrich Chemical (Milwaukee, WI, USA).

Drug-evoked \( ^{3} \text{H} \)-HT release. Outdated human platelets were obtained from the blood bank at Pitt County Memorial Hospital, Greenville, NC, USA. Platelets from 10 donors were pooled, dimethylsulfoxide was added to 10% volume, and 6 ml aliquots were stored frozen at \(-80^{\circ}\) until use. To assess drug-evoked \( ^{3} \text{H} \)-HT release, an aliquot of frozen platelets was thawed and suspended in 10 ml ice-cold Krebs-Ringer-HEPES (KRH) buffer as previously described (Cozzi & Foley 2002). The KRH buffer contained the following ingredients: 124.0 mM NaCl, 2.9 mM KCl, 1.3 mM MgSO_4, 1.2 mM KH_2PO_4, 2.4 mM CaCl_2, 5.2 mM d-glucose, 25.0 mM HEPES, 0.1 mM sodium ascorbate, 0.1 mM pargyline. The buffer was adjusted to pH 7.4 with 5M NaOH. A 5.1 µl aliquot of \( ^{3} \text{H} \)-HT (39.2 µM stock solution) was added to the platelet suspension to give a final concentration of 20 nM. The platelets were then incubated at 37° for 20 min. with shaking to allow neurotransmitter uptake. After the labeling incubation, 250 µl aliquots of the platelet suspension were transferred to each of 9 superfusion chambers with Whatman GF/B filter disks. The platelets were superfused with 37° KRH at a rate of 0.5 ml/min. for a 20 min. wash-out period to achieve a basal level of spontaneous \( ^{3} \text{H} \)-HT release. Following the washout period, 10 serial 2 min. (1 ml) superfusate fractions were collected directly into plastic liquid scintillation vials. At the end of the experiment the glass fiber filters containing the platelets were also placed into scintillation vials containing 1 ml KRH buffer. Scintillation cocktail was added (4 ml; SclintSafe 30%, Fischer Scientific, Pittsburgh, PA, USA) to all the vials and the vials were sealed and vortexed. Radioactivity was measured using a Packard Tri-Carb 2200CA liquid scintillation counter.

To test the effects of methcathinone and para-methoxyamphetamine on \( ^{3} \text{H} \)-HT release, the drugs were introduced into some of the superfusion chambers at a concentration of 10 µM in KRH during fractions 3 and 4. The amount of tritium released in the presence of drugs was compared to the amount released in the absence of drugs and is expressed as percent released. Percent released for any fraction is calculated by dividing the amount of tritium released (dpm) during that fraction by the total platelet tritium content at the start of that fraction collection period and multiplying by 100. The platelet tritium content at the start of a collection period is the sum of the tritium released during that collection period, all subsequent collection periods, and the glass fiber filter tritium content at the end of the experiment.

Cell-specific \( ^{3} \text{H} \)-methcathinone uptake. To directly assess whether methcathinone is a serotonin uptake transporter substrate we added \( ^{3} \text{H} \)-methcathinone to 293SERT cells (generously supplied by Dr. Randy Blakely, Vanderbilt University) and to wild-type HEK 293 cells (American Type Culture Collection, Rockville, MD, USA) under several conditions known to affect serotonin uptake transporter function. 293SERT cells are identical to HEK 293 cells except they stably express the human serotonin uptake transporter. 293SERT cells were maintained in a humidified atmosphere (5% CO_2 in air) in selective culture medium containing Dulbecco’s Modified Eagle’s Medium (DMEM), 10% fetal bovine serum, and antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml geneticin). The wild-type HEK 293 cells were maintained in the...
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The ability of the cells to accumulate \( [\text{H}] \)methcathinone was measured under the following conditions: at 37°, at 0°, in the presence of fluoxetine, or in sodium-free buffer. For uptake assays, 6×100-mm dishes of confluent 293SERT or HEK 293 cells were washed with ice-cold KRH buffer then dislodged with a cell scraper. Cells were suspended in ice-cold KRH or in sodium-free KRH (KRH buffer with an isoosmotic concentration of TRIS hydrochloride substituting for NaCl) by gentle pipetting. Triplicate 980 µl aliquots of the cell suspension, adjusted to contain 300 µg protein/aliquot, were added to test tubes on ice. Protein concentration was determined by the method of Bradford (1976). After adding the cell suspension, 10 µL KRH (for “37°” and “0°” determinations), 10 µL of 10 mM fluoxetine hydrochloride (for “FLX” determinations; final concentration, 100 µM), or 10 µL of sodium-free KRH (for “–Na+” assays) was added to the appropriate tubes. All tubes were preincubated in a shaking water bath at 37° for 10 min., then returned to the ice-bath for 10 min. To initiate uptake, 10 µL of 1 µM \( [\text{H}] \)methcathinone solution was added to each tube (final concentration, 10 nM) and all tubes except 0° tubes were again incubated in the 37° shaking water bath. Uptake was allowed to proceed for 10 min. before the test tubes were returned to the ice-bath. The assay tubes were filtered, processed, and counted using liquid scintillation spectrophotometry as described above. Data from 3-5 experiments were collected and transformed from dpm to fmol/min./mg protein based upon the specific activity (80 Ci/mmol) of the \( [\text{H}] \)methcathinone used.

Cell-specific \( [\text{H}] \)5-HT uptake. To confirm that 293SERT cells, but not HEK 293 cells, express functional serotonin transporters, experiments were conducted as described above except \( [\text{H}] \)5-HT was used as the transporter substrate. Cells were incubated with 10 nM \( [\text{H}] \)5-HT under several conditions (“37°”, “0°”, “FLX”), then filtered and worked-up as described above.

Kinetic parameters of \( [\text{H}] \)methcathinone uptake. To determine \( K_M \) and \( V_{\text{max}} \) values for methcathinone accumulation into 293SERT cells, we incubated cells with increasing concentrations of \( [\text{H}] \)methcathinone under conditions used to assess serotonin uptake. 293SERT cells were grown and harvested as described above and suspended in ice-cold KRH at a concentration of 612 µg protein/ml. Triplicate 490 µl aliquots of the cell suspension (300 µg protein/aliquot) were added to test tubes on ice containing either 5 µL KRH (for “total” determinations) or 5 µL of 10 mM fluoxetine (for “non-specific” determinations; final concentration 100 µM). One hundred \( \times \) solutions of \( [\text{H}] \)methcathinone were prepared from concentrated stock solution, then 5 µL of these working solutions were added to test tubes to give final concentrations ranging from 10 nM to 320 nM. The assay tubes were transferred to the 37° shaking water bath to initiate uptake. After 10 min., the tubes were returned to the ice-bath, 3 ml ice-cold KRH was added, and the contents were vacuum filtered, washed, and counted as described above. Non-specific dpm were subtracted from total dpm to calculate specific uptake. Data were transformed from dpm to fmol/min./mg protein. Data from 2–7 experiments per concentration were combined and used for curve fitting. Data were fitted using commercial computer software (GraphPad Prism, San Diego, CA, USA).

Statistics. All values are expressed as the mean±standard error of the mean (S.E.M.). Pairwise comparisons of \( [\text{H}] \)methcathinone up-
take between HEK 293 cells and 293SERT cells under each condition of temperature, inhibitor, or ion composition were made using Student's t-test. Different treatments in 293SERT cells were compared to the 37°C condition by one-way ANOVA followed by Dunnett's t-test. P<0.05 was considered significant.

Results

Results of the superfusion experiments are shown in fig. 1. Under superfusion conditions, human platelets preloaded with [3H]5-HT released a slowly decreasing amount of radioactivity over time in the absence of drugs. When 10 μM methcathinone was added to the superfusion buffer during fractions 3 and 4, a large increase in the efflux of tritium was observed. Para-methylythioamphetamine also increased the amount of radioactivity released by the superfused platelets, though not to the same extent as methcathinone (fig. 1). After the drug-containing buffers were switched back to drug-free buffer during fraction 5 and thereafter, the amount of released tritium declined and reached control levels by the end of the experiment.

Before conducting uptake experiments with [3H]methcathinone, we confirmed that 293SERT cells, but not HEK 293 cells, take up [3H]5-HT and that this uptake is temperature sensitive and is blockable by fluoxetine (data not shown). Cell-specific accumulation of [3H]methcathinone is shown in fig. 2. 293SERT cells accumulate [3H]methcathinone and this accumulation is temperature-sensitive, is inhibited by fluoxetine, and is sodium-dependent. HEK 293 cells, on the other hand, did not store [3H]methcathinone under any of the test conditions. Specific [3H]methcathinone uptake at 37°C, defined as uptake in 293SERT cells minus uptake in HEK 293 cells, was 45.0 fmol/min./mg protein. All treatments in the 293SERT cells differed from the 37°C value at P<0.05 (Dunnett's t-test). An association of [3H]methcathinone with 293SERT cells, but not HEK 293 cells, was observed under the “0°C” and “fluoxetine” treatment conditions (P<0.01; Student's t-test). This was eliminated by removing sodium ions from the incubation medium.

Serotonin transporter-mediated uptake of [3H]methcathinone was further characterized by incubating 293SERT cells with concentrations of [3H]methcathinone ranging from 10 nM to 320 nM in the absence and presence of fluoxetine. After subtracting fluoxetine-defined non-specific radioactivity, non-linear regression analysis revealed that [3H]methcathinone accumulation was saturable and was best represented by a single-site model (fig. 3). The non-linear regression coefficients for K_m and V_max were 244±51 nM and 202±25 fmol/min./mg protein, respectively. Data were also transformed for display as a double-reciprocal plot (fig. 3).

Discussion

Drugs acting at the serotonin uptake transporter can be broadly classified as non-substrate uptake inhibitors such as fluoxetine or substrate analogues such as 3,4-methylenedioxyamphetamine (MDMA). The former compounds prevent serotonin uptake but are not themselves transported into the cell. Substrate analogues, on the other hand, are capable of being translocated across the cell membrane in place of serotonin and thereby inhibit serotonin uptake by competing for the limited number of transport proteins in a cell. These drugs also cause the release of cytosolic serotonin through a serotonin uptake transporter-mediated exchange mechanism (Rudnick 1997). We previously reported that methcathinone inhibits [3H]5-HT uptake into human platelets (Cozzi et al. 1999), but under those experimental conditions we could not distinguish whether the drug was a pure uptake inhibitor or whether it was a substrate for the serotonin uptake transporter. To answer this question and to further elucidate the mechanism of action of methcathinone we tested the hypothesis that methcathinone is a substrate for the serotonin uptake transporter.

Human platelets contain serotonin transporter proteins and are a long-accepted model for serotonin transport across the cell membrane. After preloading human platelets with [3H]5-HT and then superfusing them with 10 μM methcathinone, there was a 3-fold peak increase in the amount of radioactivity released into the superfusate (fig. 1). These results are consistent with the hypothesis that methcathinone is a serotonin uptake transporter substrate because under the experimental conditions described, if a substrate is present in the superfusion buffer, it will be taken...
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Fig. 3. Accumulation of \([3H]\)methcathinone by 293SERT cells. 293SERT cells were incubated for 10 min. with increasing concentrations of \([3H]\)methcathinone under conditions used to assess neurotransmitter uptake. Data are the mean of 2–7 experiments per concentration, each performed in triplicate. Non-specific uptake was defined by 100 \(\mu M\) fluoxetine. Specific uptake was saturable and the data were best fitted, using non-linear regression, to a single-site model. For calculation of \(V_{\text{max}}\), data were converted from dpm to fmol/min./mg protein based upon the specific activity (80 Ci/mmol) of the \([3H]\)methcathinone used. Left panel: saturation isotherm; \(K_M=244\pm51 \text{nM}, \ V_{\text{max}}=202\pm25 \text{fmol/min./mg protein}\). Right panel: double reciprocal plot of specific uptake data.

To further characterize methcathinone as a serotonin uptake transporter substrate and serotonin-releasing agent (Huang et al. 1992; Scorza et al. 1999; Gobbi et al. 2002).

To further characterize methcathinone as a serotonin uptake transporter substrate we studied its accumulation into wild-type HEK 293 cells and into 293SERT cells under conditions known to affect serotonin uptake transporter function. The use of a transfected cell line to assess \([3H]\)methcathinone uptake avoids the potentially confounding effects on \(K_M\) and \(V_{\text{max}}\) caused by dopamine and norepinephrine uptake mechanisms which exist in platelets (Abrams & Solomon 1969; Dean & Copolov 1989). We first confirmed that \([3H]\)5-HT uptake was cell-specific. We then examined \([3H]\)methcathinone accumulation under several conditions known to affect serotonin uptake transporter function. We also incubated 293SERT cells with increasing concentrations of \([3H]\)methcathinone to determine \(K_M\) and \(V_{\text{max}}\) values for transport. 293SERT cells, but not HEK 293 cells, stored \([3H]\)5-HT (data not shown) or \([3H]\)methcathinone (fig. 2) under physiological conditions. Treating the HEK 293 data as non-specific, uptake of \([3H]\)methcathinone into 293SERT cells was about 63% specific (45.0 fmol/min./mg protein). When 293SERT cells were incubated at 0\(^\circ\), specific uptake was reduced about 46% compared to the 37\(^\circ\) condition (P<0.05) but was not completely abolished compared to the HEK 293 control cells (P<0.01). The inhibition of uptake by cold temperatures is thus not as robust as we have come to expect from our previous work. The remaining radioactivity could represent binding of \([3H]\)methcathinone to the serotonin uptake transporter, even though transport itself is inhibited by cold temperatures. When 293SERT cells were incubated with \([3H]\)methcathinone at 37\(^\circ\) in the presence of fluoxetine or in sodium-free buffer, the tritium associated with the cells was substantially reduced (P<0.01). Under these conditions, the amount of radioligand was not statistically different from the amount of radioligand observed in the non-transfected HEK 293 cells (fig. 2). Together, these data show that \([3H]\)methcathinone uptake is cell-specific and that methcathinone exhibits properties consistent with a serotonin uptake transporter substrate.

Kinetic constants for \([3H]\)methcathinone intake by
293SERT cells were derived from non-linear regression analysis of specific [3H]methcathinone uptake as increasing concentrations of the radioligand were added to the cell suspensions (fig. 3). The data fit significantly better to a one-site model as compared to a two-site model and the double reciprocal transformation supports this conclusion (fig. 3). Previously, we reported an IC_{50} value for methcathinone inhibition of [3H]-HT uptake into human platelets of 35 μM (Cozzi et al. 1999). The present study reports a K_M for [3H]methcathinone uptake of 244 nM in 293SERT cells. The different values for IC_{50} and K_M may reflect differences in transport kinetics between serotonin and methcathinone or between native platelets and transfected cells or differences between the mechanism of competitive uptake inhibition and the mechanism of translocation itself. If, as seems likely, intermediate conformational states of the transporter occur as part of the translocation process, the intermediate conformational states of the transporter may have their own associated rate constants for interconversion between states. Under these conditions transport will not follow simple Michaelis-Menten kinetics and K_M will not equal the equilibrium dissociation constant implied by the IC_{50} (Fersht 1985). Various kinetic constants have been reported for the uptake of serotonin itself into 293SERT cells. For example, the K_M and V_{max} values for [3H]-HT uptake into 293SERT cells have been reported to range from 260 to 600 nM and from 11.9 to 1377 pmol/min./10^6 cells, respectively (Qian et al. 1997; Sitte et al. 2001). The range of reported K_M and V_{max} values apparently reflects variations in serotonin uptake transporter expression and activity as cells are maintained in culture for extended periods.

Several research groups have reported that methcathinone produces decreases in dopamine and serotonin markers in rat brain (Gygi et al. 1996 & 1997; Sparago et al. 1996; Metzger et al. 1998; Fleckenstein et al. 1999). If these decreases result from the same mechanism responsible for the neurotoxic effects of related drugs such as methamphetamine, para-chloroamphetamine, and MDMA, then a key step in methcathinone-induced neurotoxicity is transport of the drug into the cell via the plasma membrane monoamine uptake transporters. Although evidence exists that methcathinone is a substrate for the dopamine transporter (Glennon et al. 1987; Gygi et al. 1997), no data existed for similar activity at the serotonin uptake transporter prior to this report. Our results indicate that methcathinone is a substrate for the serotonin uptake transporter and extend our understanding of the mechanism of action of this psychostimulant drug. Neuronal uptake of methcathinone by the serotonin uptake transporter may be an essential step in the production of serotonergic neurotoxicity. If so, blocking the serotonin uptake transporter with drugs such as fluoxetine may reduce the potential for serotonin neurotoxicity.

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References
Fersht, A.: Enzyme structure and mechanism, 2nd ed. W. H. Free
McCann, U. D., D. F. Wong, F. Yokoi, V. Vleminge, R. F. Dan
lows & G. A. Ricaurte: Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathinone users:
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