



Effects of 5-HT-releasing agents on the extracellular hippocampal 5-HT of rats. Implications for the development of novel antidepressants with a short onset of action

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Abstract

The effects of two selective 5-HT-releasing agents, 4-methylthioamphetamine (MTA) and 5-methoxy-6-methyl-2-aminoindan (MMAI), on the extracellular 5-HT concentration in the dorsal hippocampus was determined by microdialysis in anesthetized rats. After i.p. administration of 1 or 5 mg/kg of either compound, a rapid and significant increase of 5-HT basal release was observed. MTA (5 mg/kg) induced a maximal increase of about 2000% over the basal value 40 min after injection, which declined slowly, whereas MMAI (5 mg/kg) induced a maximal response of about 1350% which showed a rapid decline. Monoamine oxidase-A inhibitory properties of MTA, and MMAI's lack of similar properties might account for the difference between the two compounds. In agreement with previous information, a much lower increase in hippocampal 5-HT was observed in response to systemic fluoxetine. This difference in the magnitude of the response after MTA or MMAI and fluoxetine indicates that different mechanisms of action are operating. Based on evidence showing that an acute enhancement of 5-HT neurotransmission might result in the rapid appearance of therapeutic effects of serotonergic antidepressants, we suggest that MTA and MMAI might serve as leads for a novel family of compounds with a short onset of action useful for treating depression. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Several lines of evidence indicate that an enhancement of serotonin (5-HT) neurotransmission might underlie the therapeutic response to different types of antidepressant treatments (Blier and de Montigny, 1994). Although the primary neurochemical actions of antidepressant drugs such as selective 5-HT reuptake inhibitors (SSRIs) or monoamine oxidase-A inhibitors (MAOIs-A) are present within a few minutes, the onset of their therapeutic effects requires from one to two

weeks of treatment (Danjou et al., 1994). Adaptive changes have been proposed to explain this paradoxical effect. Electrophysiological and microdialysis studies have shown that acute systemic administration of antidepressants leads to a decrease in the firing rate of 5-HT neurons that is postulated to be due to negative feedback produced by the activation of 5-HT_{1A} somatodendritic autoreceptors located in the raphe nuclei (Blier et al., 1990; Invernizzi et al., 1992; Bel and Artigas, 1993; Hjorth, 1993; Gartside et al., 1995; Hjorth et al., 1996). Desensitization of these receptors after chronic antidepressant treatment and the consequent recovery of the serotonergic firing rate might explain, at least in part, the delayed onset of action of SSRIs and MAOIs-A (Blier and de Montigny, 1994).

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At present, different pharmacological strategies are being developed in an attempt to increase 5-HT neurotransmission more rapidly, and thus shorten the onset of action of antidepressants. For example, combined treatment with a SSRI and pindolol, a nonselective 5-HT_{1A} antagonist (Romero et al. 1996a), or the newly developed 5-HT_{1A} antagonist WAY 100635 (Gartside et al., 1995; Gundlach et al., 1997a; Hjorth et al., 1997), increases the extracellular concentration of 5-HT more than treatment with the SSRI alone, as measured by *in vivo* microdialysis in rats. Indeed, combined treatments (using pindolol as the 5-HT_{1A} antagonist) markedly reduced the latency of antidepressant response in previously untreated patients and induced a rapid improvement in treatment-resistant patients (Artigas et al., 1996; Perez et al., 1997; Tome et al., 1997; Zanardi et al., 1997; see Blier and Bergeron, 1998 for a review).

With the idea of seeking pharmacological alternatives that might rapidly increase 5-HT neurotransmission, we investigated the effects of two potent and selective 5-HT-releasing agents, 4-methylthioamphetamine (MTA) and 5-methoxy-6-methyl-2-aminoindan (MMAI) (Johnson et al., 1991; Huang et al., 1992; Nichols et al., 1993), on the extracellular 5-HT concentration in the dorsal hippocampus using *in vivo* microdialysis in anesthetized rats. In addition, as MAO-A inhibitory properties have been reported for MTA (Scorza et al., 1997), we also evaluated the effects of MMAI upon MAO activity.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats weighing 260–310 g were used. They were kept, six per cage, in a controlled environment with a 12 h light–dark cycle and 21°C room temperature. Food and water were provided *ad libitum*.

2.2. Drugs

MTA and MMAI (both as hydrochloride salts) were synthesized in DENs laboratory. Fluoxetine was donated by Eli Lilly and Co. (Indianapolis, IN). Drugs were dissolved in saline and were injected *i.p.* in volumes of 1 ml/kg.

2.3. Microdialysis procedure

Since microdialysis in anesthetized animals is a well validated methodological approach (Ungerstedt, 1991) and is particularly suitable for the screening of pharmacological effects of novel drugs, we chose this modality to evaluate the effects of MTA and MMAI. Rats,

anesthetized with urethane (3 g/kg *i.p.*) were mounted in a stereotaxic frame (David Kopf), and a hole was drilled in the skull to allow implantation of a microdialysis probe (dialyzing length 2 mm, diameter 0.5 mm, CMA-Microdialysis AB, Stockholm, Sweden).

The probe was implanted into the dorsal hippocampus (coordinates with respect to bregma: AP = −3.8, L = −2.6, DV = −3.6, according to Paxinos and Watson, 1986) and was perfused with artificial cerebrospinal fluid (in mM: NaCl, 148; KCl, 2.75; CaCl₂, 1.2; MgCl₂, 0.85; pH = 6.8) at a rate of 0.6 µl/min using a microinjection pump (CMA/100 micropump). Samples were collected every 20 min (total volume = 12 µl; analyzed volume = 10 µl).

After stabilization of baseline 5-HT levels (120–180 min post probe implantation) three fractions were collected to assess the basal 5-HT release before the systemic administration of drugs. At the end of each experiment 100 mM KCl was perfused to confirm that synaptic functionality was preserved.

Animals were placed on a heating pad to maintain body temperature close to 37°C.

2.4. Neurochemical assay

The content of 5-HT in dialysates was analyzed by HPLC with electrochemical detection. The HPLC-EC system was a BAS LC-4C (Bioanalytical Systems, West Lafayette, IN, USA) equipped with a Unijet Microbore column (3 µm-C₁₈ 100 × 1 mm) and a 3 mm Unijet radial flow, glassy carbon thin-layer electrochemical cell. These conditions enhance the detectability of 5-HT basal levels and make the perfusion of a 5-HT reuptake-inhibitor unnecessary. The maximum sensitivity used with the amperometric detector was 0.5 nA and the oxidation potential was set at 0.65 V versus a Ag/AgCl reference electrode. The mobile phase flow rate was 0.5 ml/min and its composition was 31.5 g citric acid; 930 ml twice-distilled water; 584 mg NaCl; sufficient 12.5 M NaOH to bring the pH value to 3; 200 mg sodium octyl sulfate; 34 ml acetonitrile and 33 ml THF. Under these experimental conditions the detection limit for 5-HT was 1–1.5 fmol/10 µl sample (signal:noise ratio = 2:1). After each experiment, animals were sacrificed and the placement of the cannulae was verified by histological examination. Animals with tissue damage, brain edema or not responding to KCl stimulation were excluded from data groups.

2.5. MAO assay

The effect of MMAI on MAO activity was studied using a crude rat brain mitochondrial suspension as the source of enzyme. The mitochondrial MAO activities were determined by HPLC-EC using selective substrates for MAO-A (5-HT) and MAO-B (4-dimethyl-

aminophenethylamine) following a previously published procedure (Scorza et al., 1997).

2.6. Statistics

Microdialysis results are expressed as femtomoles per fraction (uncorrected for recovery) and represented (mean \pm SEM) as percentage of basal values. Statistical analysis was performed using one-way repeated-measures analysis of variance (ANOVA) followed by the Dunnett's Multiple Comparisons test. Significance was set at $P < 0.05$.

3. Results

Under the experimental conditions used, the mean \pm SEM 5-HT basal value was 3.3 ± 0.8 fmol/10 μ l ($n = 35$). This value is similar to that reported by different authors using chloral hydrate anesthetized rats (see for example Hjorth et al., 1996; Gartside et al., 1997) or awake animals (see for example Invernizzi et al., 1997; Romero and Artigas, 1997).

Either MTA or MMAI induced an acute and dose-dependent increase of extracellular 5-HT in the dorsal hippocampus (Fig. 1 A, B). One-way ANOVA showed a significant effect of MTA at both doses tested [$F = 10.96$, $P < 0.0001$ (5 mg/kg)] and [$F = 3.14$, $P < 0.05$ (1 mg/kg)]. Post hoc comparisons done with the Dunnett test indicated that the 5 mg/kg dose induced a significant and sustained increase of hippocampal 5-HT starting 40 min after drug administration ($P < 0.01$), whereas the effect of 1 mg/kg was significant only 80 min after drug injection ($P < 0.05$) (Fig. 1A).

Similarly, in the case of MMAI, one-way ANOVA showed a significant effect of both doses [$F = 34.22$, $P < 0.0001$ (5 mg/kg)] and [$F = 4.13$, $P < 0.01$ (1 mg/kg)]. Post hoc comparisons indicated that 5-HT values after 5 mg/kg of MMAI were significantly different from basal values ($P < 0.01$ and $P < 0.05$). The 1 mg/kg dose induced a significant increase only 60 and 80 min after drug administration ($P < 0.01$ and $P < 0.05$ respectively) (Fig. 1B).

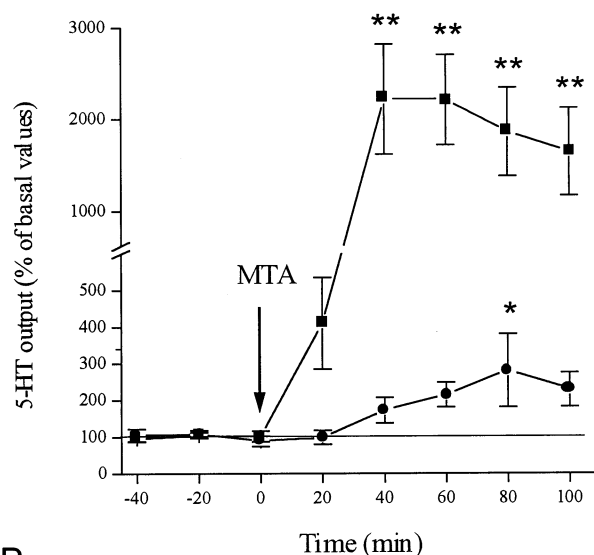
In order to compare the effect of these 5-HT releasing agents with a known SSRI, we determined the effect of fluoxetine on hippocampal 5-HT under the same experimental conditions described above. As shown in Fig. 2, the i.p. administration of fluoxetine (10 mg/kg) induced a significant increase of extracellular 5-HT starting 40 min after i.p. injection [$F = 5.31$, $P < 0.001$].

The 20 min pulse of 100 mM KCl administered through the cannula at the end of each experiment evoked an immediate and significant increase of 5-HT release which ranged from 1.5–2.8-fold in the case of MTA and MMAI to 5.6-fold in the case of fluoxetine treated animals, as compared with the value observed

immediately before the KCl perfusion (data not shown). In the whole experiment only two animals (out of 37) not responding to KCl were discarded, and it should be noted that even in those instances where 5-HT release remained high (as in the case of animals treated with MTA 5 mg/kg) a significant increase was still elicited by KCl perfusion.

Fig. 3 shows the effects of MMAI and MTA upon MAO activity. The MAO-A inhibitory effect of MMAI

A



B

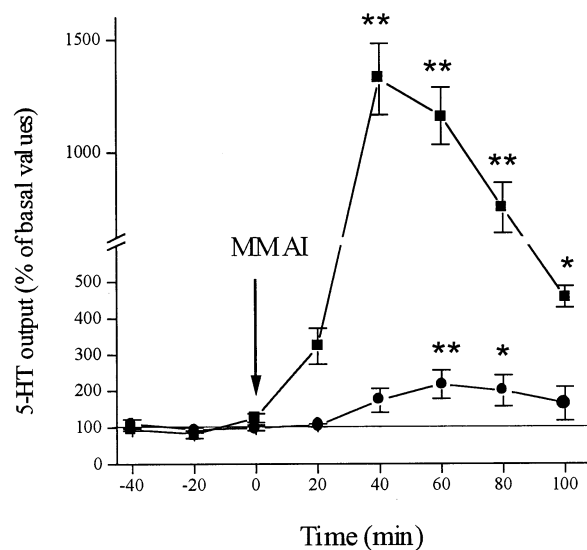


Fig. 1. Effects of MTA (A) and MMAI (B) on 5-HT extracellular concentration in the dorsal hippocampus as measured by microdialysis in rats. Data are given as means \pm SEM of five to six animals. Systemic administration of drugs (1 mg/kg = circles; 5 mg/kg = squares) is shown by the arrow. Asterisks denote values significantly different from basal (* $P < 0.05$, ** $P < 0.01$, Dunnett test after significant ANOVA for repeated measures).

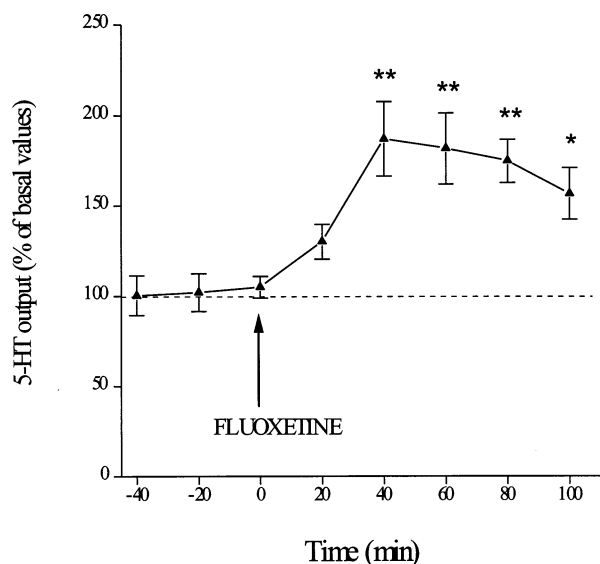


Fig. 2. Effect of fluoxetine on 5-HT extracellular concentration in the dorsal hippocampus as measured by microdialysis in rats. Data are given as means \pm SEM of nine animals. Systemic administration of drug (10 mg/kg) is shown by the arrow. Asterisks denote values significantly different from basal (* P < 0.05, ** P < 0.01, Dunnett test after significant ANOVA for repeated measures).

was clearly less than that reported for MTA. In addition, MMAI was completely devoid of MAO-B inhibitory properties.

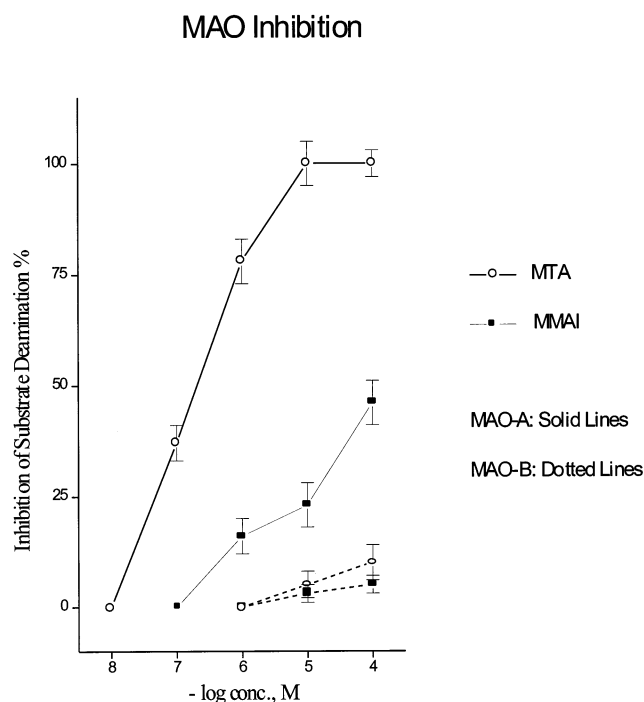


Fig. 3. Effects of MTA (○) and MMAI (■) as MAOIs. Inhibition of MAO-A (solid lines) and MAO-B (dotted lines) is represented as the percentage of inhibition of deamination of 5-HT (for MAO-A) and 4-dimethylaminophenethylamine (for MAO-B), as measured by HPLC-EC. Each point is the mean \pm SD of three determinations.

4. Discussion

In the present work systemic administration of either MTA or MMAI, two very selective and non-neurotoxic 5-HT releasers (Johnson et al., 1991; Huang et al., 1992; Nichols et al., 1993), produced a rapid and very marked increase of extracellular 5-HT levels in the dorsal hippocampus. Thus, our results confirm in vivo the 5-HT-releasing properties of MTA and MMAI described in earlier in vitro experiments. The magnitude of this increase, as well as its marked dose-dependence, are similar to the responses observed in different CNS regions after acute administration of other 5-HT-releasing agents such as *p*-chloroamphetamine (PCA) (Sharp et al., 1986; Hutson and Curzon, 1989) *D,L*-fenfluramine (Gundlah et al., 1997b), *D*-fenfluramine (Schwartz et al., 1989; Puig de Parada et al., 1995; Rocher et al., 1996), *D*-norfenfluramine (Puig de Parada et al., 1995) or methylenedioxymethamphetamine (MDMA) (Gartside et al., 1997). Conversely, in agreement with findings from several laboratories (see Fuller, 1994 for a review), a much smaller increase in hippocampal 5-HT release was observed in response to systemic fluoxetine. As Gundlah et al. (1997b) have previously discussed, the difference in the magnitude of the response after MTA or MMAI and fluoxetine indicates that different mechanisms of action are operating.

Differential time courses were noted in response to MMAI and MTA. While in the case of MMAI there was a rapid decline after the maximal response was attained, MTA induced a larger and more sustained increase of extracellular 5-HT. Similar results to those obtained with MMAI have been reported using other 5-HT releasing agents (Puig de Parada et al., 1995; Gartside et al., 1997; Gundlah et al., 1997b), an effect that might be related to a partial depletion of the releasable pool (Mück-Seler and Diksic, 1996; Gundlah et al., 1997b). The MAO-A inhibitory properties of MTA (and MMAI's lack of similar properties) might account for the difference between the two compounds studied here. It has been shown that amphetamine derivatives such as MMAI, MDMA or PCA stimulate 5-HT release by a process mediated by the 5-HT transporter which possibly involves exchange diffusion of 5-HT (Rudnick and Wall, 1992a,b, 1993). In addition, as in the case of other indirectly acting amines (Langeloh et al., 1987; Langeloh and Trendelenburg 1987; Ask et al. 1989), it has been suggested that these compounds might mobilize 5-HT from their intracellular storage vesicles into the axoplasm which would increase the availability of the transmitter to be released, in a process presumably involving intravesicular pH increase (Rudnick and Wall, 1992a,b, 1993). In this context, Langeloh and Trendelenburg (1987) have pointed out that transmitter mobilized from vesicles

close to the membrane has better chances of being released than that coming from more internal vesicles, because of the higher likelihood of the latter of being metabolized intracellularly by MAO. Thus, while in the case of MMAI the 5-HT released could be (intra and extracellularly) metabolized, the MAO-A inhibition induced by MTA might be responsible for the higher and sustained increase of 5-HT extracellular levels after treatment with this drug. Accordingly, increased tissue contents of 5-HT and a reduction of 5-hydroxyindolacetic acid have been observed in the hippocampus for at least 6 h after systemic administration of MTA (Scorza et al., 1997). On the other hand, it has been suggested that MTA might be a neuron-selective MAO-A inhibitor similar to amiflamine (Ask et al., 1985; Scorza et al., 1997), i.e. this compound would preferably inhibit MAO-A inside 5-HT neurons after being transported and concentrated into these cells. Although a much weaker MAOI-A, MMAI might also be concentrated into 5-HT neurons to such an extent that MAO were inhibited, and this possibility should be considered when analyzing the effects of this releasing agent.

Although methodological differences must be considered, it may be seen that maximal responses observed after MTA and MMAI at 5 mg/kg were similar, although greater than those obtained with the combination of SSRIs or MAOIs and 5-HT_{1A} antagonists (Romero et al., 1996a,b; Gundlah et al., 1997b; Hjorth et al., 1997). In spite of the auspicious results obtained in humans with treatments combining the use of a SSRI and pindolol, a 5-HT_{1A} antagonist that apparently blocks only somatodendritic 5-HT_{1A} autoreceptors (Romero et al., 1996a), the blockade of 5-HT_{1A} receptors in order to shorten the onset of action of antidepressants, as a therapeutic concept, may be problematic. Therapies based on the administration of mixtures of drugs are, at the least, subject to difficulties arising from differing dose-response relationships and pharmacokinetics of the individual components.

It might be noted further that facilitation of 5-HT_{1A}-mediated neurotransmission in limbic regions such as the hippocampus has been implicated as one of the critical events in attenuating behavioral consequences of stress (Graeff et al., 1996) and also seems to play an important role in obtaining an antidepressant response (Blier et al., 1997). Thus, a possible option to enhance this effect might be the use of compounds that release 5-HT from its neuronal stores. Several drugs such as fenfluramine, PCA or MDMA are known to act by this mechanism (Nichols, 1994). Nevertheless, their reported lack of specificity and selective serotonergic neurotoxicity (Nichols et al., 1993) preclude their consideration as candidates for further evaluation. Thus, the use of novel non-neurotoxic 5-HT releasers appears as a plausible alternative. In addition, it should be noted that

fenfluramine, which has been reported as having neither antidepressant properties (Lichtemberg et al., 1992) nor the ability to potentiate the effects of tricyclic antidepressants (Price et al., 1990), seems to release 5-HT preferentially from terminals originating in the dorsal raphe (Viana et al., 1996; see also the magnitude of the release reported by Stancampiano et al., 1997). Thus, although further studies are necessary to clarify if these compounds differentially affect inputs arising from dorsal raphe or median raphe nuclei, the effects of MTA and MMAI in the dorsal hippocampus, which receives 5-HT innervation preferentially from median raphe (McQuade and Sharp, 1997), are promising in terms of an antidepressant effect.

These arguments are further strengthened by the recent observation of antidepressant-like activity for both MMAI and MTA in the chronic mild stress model of depression in rats (Marona-Lewicka and Nichols, 1997) and in the forced swimming test in mice (Reyes-Parada et al., unpublished observations). It is noteworthy that the doses selected in the present study were identical to those showing antidepressant effects in behavioral paradigms. It could be argued that, in the case of MTA, the antidepressant action observed in behavioral models might be attributed in part to its MAO-A inhibitory properties. However, the minimal effect of MMAI on MAO activity suggests that, although MAO-A inhibition may contribute to the overall effect of MTA, 5-HT release is the primary mechanism responsible for the antidepressant activity of these compounds.

Nevertheless, a word of caution should be offered regarding the possible therapeutic usefulness of this class of compounds. Some behavioral signs of the 5-HT syndrome have been observed in rats after the administration of relatively high doses of either MMAI or MTA (Huang et al., 1992; Marona-Lewicka and Nichols, 1994). It has been reported that a marked increase of 5-HT as a consequence of administering a MAOI-A/SSRI combination for instance, might induce a 'serotonin syndrome' in humans as well (Sternbach, 1991). The possibility of this severe adverse effect developing must be considered, particularly in the case of MTA, if the therapeutic usefulness of these compounds is to be assessed.

In addition, it should be noted that tachyphylaxis is a common feature of indirectly acting amines after repeated administration (Lefkowitz et al., 1996). As the effects of long term treatment with MTA and/or MMAI have not yet been studied, any consideration of their possible value as therapeutic agents must await studies addressing this issue. Finally, although a remarkable *in vitro* serotonergic selectivity has been reported for these compounds, *in vivo* interactions with noradrenergic and/or dopaminergic systems cannot be ruled out at this time since they are amphetamine

derivatives. Further studies are necessary to clarify these points.

Despite these considerations, it is worth pointing out that the results presented here indicate that both MTA and MMAI appear to be useful tools to study the role of 5-HT, and its acute increase in the synaptic cleft, in different physiological and pathological conditions, such as in models of anxiety or depression, as well as its interactions with other neurotransmitter systems.

At present, the most sought for breakthrough in the treatment of depression is the discovery of drugs producing a rapid response. Assuming that a rapid increase of 5-HT in serotonergic synapses might lead to a short onset of antidepressant action, these and other selective and non-neurotoxic 5-HT releasing agents might serve as important leads for a completely novel family of compounds useful for treating depression.

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