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# DISTRIBUTION OF $D_2$ DOPAMINE RECEPTOR mRNA IN THE PRIMATE BRAIN

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## Abstract

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1. The distribution of the messenger RNA (mRNA) encoding the D2dopamine receptor has been mapped in the monkey brain by *in situ* hybridization.
2. Using [$^{35}$S]-labelled riboprobes corresponding to the region of the $D_2$ dopamine receptor spanning the third cytosolic loop and the sixth and seventh transmembrane domains, specific hybridization was observed in a number of neural structures.
3. High levels of mRNA expression were observed in the caudate, putamen, and claustrum. Significant amounts were also identified in the hippocampus, lateral geniculate nucleus, much of the cortex, amygdala, pons, and thalamus. High levels of this mRNA were also visualized in the substantia nigra, likely reflecting autoreceptor synthesis.
4. While the distribution of $D_2$ dopamine receptor mRNA was similar between the monkey and previously published maps in the rat, several differences were noted.
5. These results demonstrate the feasibility of visualizing this mRNA in the primate brain, and suggest that a similar analysis of human postmortem brain material may be possible.

Keywords: brain chemistry, catecholamines, central nervous system, dopamine receptors, messenger RNA

Abbreviations: dopamine (DA), messenger RNA (mRNA)

## Introduction

Dopamine is a key brain neurotransmitter, associated with a number of neuropsychiatric disturbances, especially schizophrenia and Parkinson disease. Several of the dopamine receptors have been recently cloned (Bunzow et al 1988, Grandy et al 1989, Dearry et al 1990, Monsma et al 1990, Sunahara et al 1990, Zhou et al 1990) and investigations related to the distribution and regulation of the messenger RNA (mRNA) encoding these receptors are now possible. The cloning of a rat $D_2$ receptor was originally reported by Bunzow and coworkers (1988), using the hamster $\beta$2-adrenergic receptor gene as a probe. A number of groups have more recently reported that there are at least two distinct forms of $D_2$ receptor mRNA (Dal Toso et al 1989, Giros et al 1989, Monsma et al 1989, Selbie et al 1989), due to alternate splicing of this gene during transcription. The original clone that was reported by Bunzow et al (1988) is 87 bases (29 amino acids) shorter than the second form that was subsequently identified; the difference between these two forms is due to the inclusion or exclusion of exon 5 in the final transcripts.

We and others have previously reported the distribution in the rat brain of the mRNA encoding the $D_2$ receptor using *in situ* hybridization (Meador-Woodruff et al 1989, Mengod et al 1989, Najlerahim et al 1989, Weiner and Brann 1989, Mansour et al 1990). In this report, we extend our previous work to include the mapping of the distribution of total $D_2$ receptor mRNA in the monkey brain. These results demonstrate the feasibility of visualizing this mRNA in the primate brain, thus lay the framework for subsequent investigations of the

nature of $D_2$ receptor gene expression in postmortem brains derived from individuals that had suffered from a variety of neuropsychiatric disorders.

## Methods

### Animals

Brains of old-world monkeys (macaca mulatta) were removed following ketamine anesthesia and cut into 1-2 cm coronal slabs, which were frozen in isopentane (-30°C) for 120 sec. Frozen tissue was cryostat-sectioned (20 um) and thaw-mounted onto microscope slides. These sections were maintained at -80°C until the time of hybridization.

### Experimental Procedure

The determination of total $D_2$ receptor mRNA was made using [$^{35}$S]-labelled riboprobes synthesized from a Sac I-Bgl II fragment of a rat $D_2$ receptor cDNA (Bunzow et al 1988). This probe is a 495 base region corresponding to the third cytosolic loop and the sixth and seventh transmembrane domains of the rat $D_2$ receptor. This region was selected as the greatest degree of dissimilarity between the catecholamine receptors occurs in the third cytosolic loop, thus providing a greater degree of probe specificity. This probe is 3' to the exon encoding the 29 amino acids which distinguish $D_{2\alpha}$ from $D_{2\beta}$ mRNAs, hence recognizes both forms, or total $D_2$ receptor mRNA. The in situ hybridization protocol that we previously described (Meador-Woodruff et al 1989, Mansour et al 1990) was used, except hybridization took place in 50% formamide buffer, and the final post-hybridization wash (60 min) was at 55°C in 0.5X SSC (300 mM NaCl/30 mM sodium citrate, pH 7.2).

## Results

$D_2$ receptor mRNA was visualized in a number of structures, including traditional dopamine terminal fields associated with both motor and limbic circuits, as well as in dopamine-containing cell bodies. A high labelling density was observed in components of the basal ganglia: the caudate, putamen, and claustrum all had high concentrations of $D_2$ receptor mRNA, although the globus pallidus had very low levels (Fig 1). A number of limbic structures were identified as having moderate levels of this message, including much of the cortex (both superficial and deep layers), the lateral septum, and the amygdala (Figs 1, 2, and 4). The hippocampus manifested distinct patterning (Fig 4): the highest density of labelling was seen in granular cell layer of the dentate gyrus, with moderate signal in the pyramidal cell layer of CA2, CA3, and CA4, with faint labelling in CA1. In addition, faint labelling was appreciated in the stratum lacunosum-moleculare. A number of other structures were identified as containing $D_2$ receptor mRNA including the lateral geniculate nucleus and the pons, while a faint homogeneous labelling could be appreciated in the thalamus (Figs 2 and 4).

$D_2$ receptor mRNA was also visualized in the midbrain dopamine-containing cell group, the pars compacta of the substantia nigra (Fig 3). This likely represents autoreceptor-encoding mRNA, which is similar to the finding of this mRNA in the midbrain dopamine-containing nuclei of the rat.

The specificity of this labelling was confirmed by several standard control experiments commonly used in in situ hybridization studies. Adjacent sections were probed with the "sense" strand riboprobe generated from the same plasmid construct used to generalte the "antisense" probe used for the images presented in theses studies, as we have previously described in our studies in the rat brain (Meador-Woodruff et al 1989, Mansour et al 1990). Additionally, some sections were treated with RNase A prior to hybridization with the "antisense" riboprobe. RNase pretreatment eliminated all of the labelling seen in the figures contained in this report. "Sense"-strand hybridization resulted in some faint labelling in some regions of the cortex and in the hippocampus, but was much less than the signal seen with the "antisense" probe, suggesting that most of the labelling seen with the "antisense" probe was specific for $D_2$ receptor mRNA. Northern gel analysis was performed with tissues corresponding to the monkey sections shown in this report, resulting in a single, uniformly-sized mRNA band in the caudate, hippocampus, and substantia nigra (data not shown).

Finally, strong labelling was observed in the cerebellum which was eliminated with RNase pretreatment, but the intensity of labelling was approximately equal between the "sense" and "antisense"-strand probes, and no mRNA band was appreciated on a Northern gel, suggesting that this cerebellar labelling was in large part artifactual.

## Discussion

These findings demonstrate that $D_2$ receptor mRNA is distributed in both traditional dopaminoceptive as well as DA-containing regions of the primate brain, and is strikingly similar to the distribution of this mRNA in the rodent brain (Meador-Woodruff et al 1990, Mengod et al 1989, Najlerahim et al 1989, Weiner and Brann 1989, Mansour et al 1990). Several differences in the distribution of this mRNA between rat and the monkey were noted, however, especially the lamination of message in the cortex and in the hippocampus. The vast majority of $D_2$ receptor mRNA in the rat cortex is in superficial layers (Mansour et al 1990), whereas in the monkey it appears in both superficial and deep layers. In the rat hippocampus, this mRNA is equally distributed throughout the pyramidal cells of CA1-CA4 and in the granular cells of the dentate gyrus (Meador-Woodruff et al 1989, Mansour et al 1990). In the monkey, the relative density of labelling is considerably more variable between these structures; in addition,

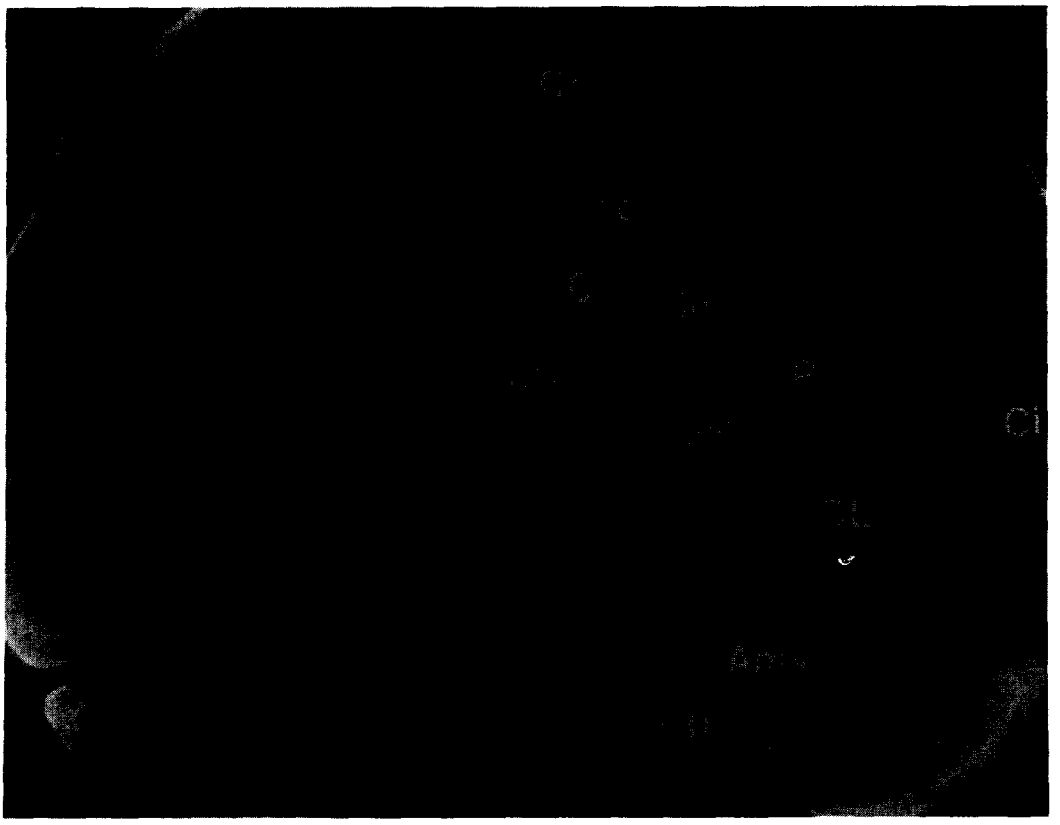


Fig.1. Distribution of $D_2$ dopamine receptor mRNA in the monkey forebrain. Note prominent labelling in the caudate (C) and putamen (P), as well as in many regions of cortex (insular cortex, Ci; cingulate cortex, Cc; piriform dortex, Cp). There is also some labelling observable in the lateral septum (LS), amygdala (Amy), and claustrum (CL). There is minimal labelling in the globus pallidus (GP).

$D_2$ receptor mRNA could also be appreciated in the stratum locunosum-moleculare, an area associated with $D_2$ receptor binding sites but not mRNA localization in the rat. The functional significance of these differences remain to be elucidated.

The localization of total $D_2$ receptor mRNA in the monkey, as well as in the rodent, parallels what would be predicted based on pat autoradiographic and immunohistochemical research identifying dopaminergic circuits in the brain (Boyson et al 1986, Bouthenet et al 1987, Charuchinda et al 1987, Richfield et al 1987, Dawson et al 1988, Wamsley et al 1989). A number of studies in the rat (Meador-Woodruff et al 1989, Mengod et al 1989, Najlerahim et al 1989, Weiner and Brann 1989, Mansour et al 1990) have demonstrated $D_2$ receptor mRNA in every region traditionally implicated as a DA receptive field, reflecting postsynaptic receptor synthesis. These studies provide anatomical confirmation of the dopaminoceptive nature of these various limbic and striatal regions.

The finding of $D_2$ receptor mRNA in some DA-containing cell groups in the rat brain (i.e.,

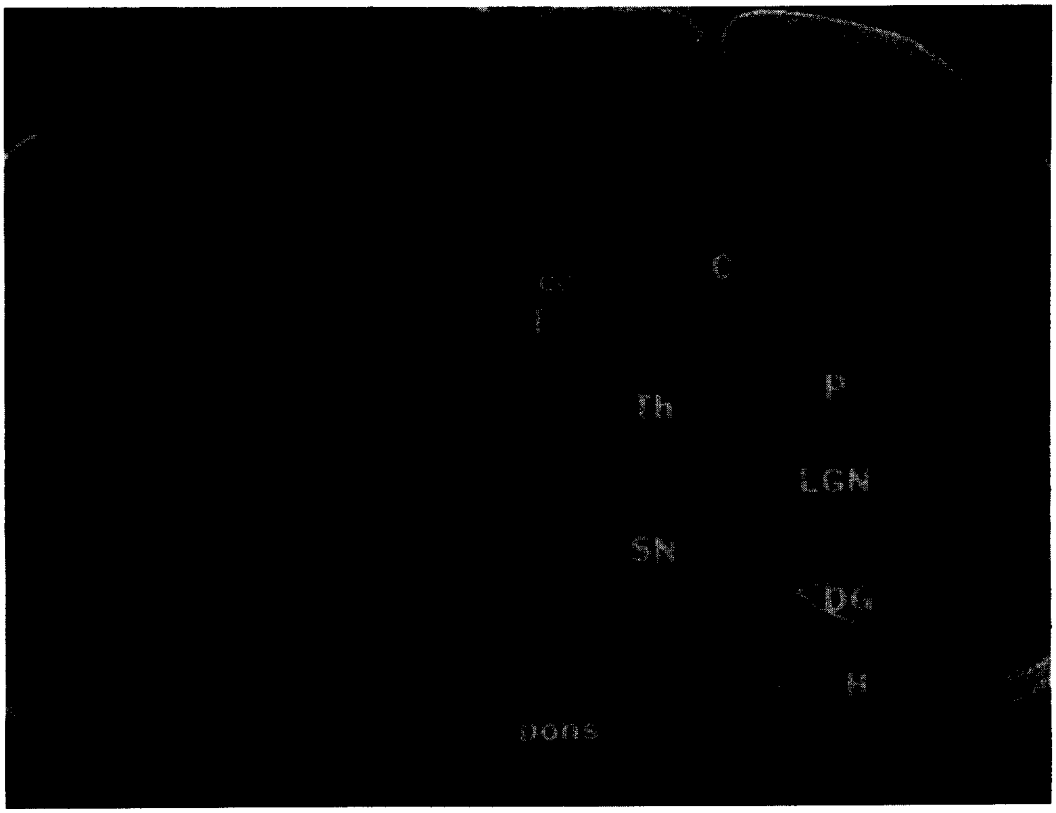


Fig 2. Distribution of $D_2$ dopamine receptor mRNA in the monkey brain at the level of the thalamus (Th). The tails of the caudate (C) and putamen (P) are visible and labelled. The substantia nigra (SN) is prominent in this section; the labelling observed in the SN probably reflects $D_2$ autoreceptor sysnthesis. Labelling is also seen in much of the cortex, as well as in the lateral geniculate nucleus (LGN), hippocampus (H) and dentate gyrus (DG), and pons. Faint labelling is seen in the thalamus.

the substantia nigra) by a number of groups (Meador-Woodruff et al 1989, Mengod et al 1989, Weiner and Brann 1989, Mansour et al 1990) suggests that D2 receptors may have an autoreceptor function. The appearance of D2 receptor mRNA in the monkey substantia nigra compliments these previous findings in the rat brain. These findings are consistent with previous studies indicating that the midbrain DA autoreceptor is D2 in nature (Reisine et al 1979, Stoof et al 1982, White and Wang 1983, Brown et al 1985).

In addition to this extension of our understanding of the brain DA systems, these studies have also demonstrated the feasibility of examining the expression of the D2 receptor gene in an anatomical context in the primate brain, thus setting the stage for postmortem investigations in the human. Messenger RNA levels tend to be quite stable in the brain following death, requiring hours to days to change appreciably in concentration (Birnberg et al 1983). Thus, mRNA levels tend not to be altered rapidly at the time of death, unlike neurotransmitters, which may be acutely released, causing changes in receptor occupancy. In addition,

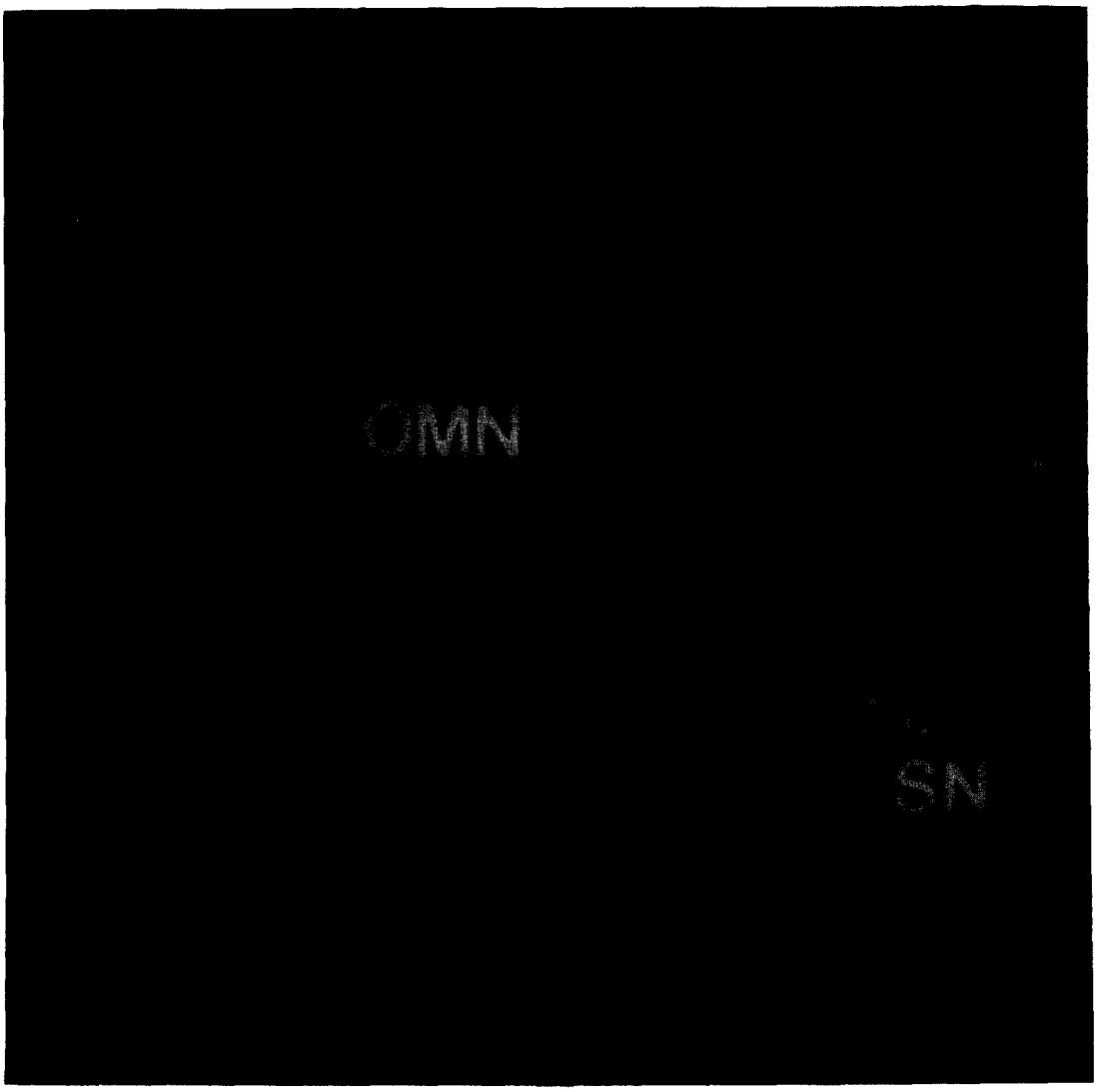


Fig 3. High power view of D2 receptor mRNA at the level of the substantia nigra (SN). In addition to strong labelling in the SN, faint labelling can be appreciated in the oculomotor nucleus (OMN, upper left corner).

the concentration of a specific mRNA in the brain likely reflects the cumulative history of the particular system rather than the more recent terminal events, thus providing a more integrated view of the regulation of a given system. Accordingly, the measurement of mRNA levels in postmortem human brain may be advantageous in the study of certain neuropsychiatric illnesses.

The present data demonstrates the feasibility of studying $D_2$ receptor mRNA in postmortem monkey brain, and hopefully reflects the level of information available from the study of the human brain. The study of the expression of the $D_2$ receptor gene in human brain should now be possible in a number of neuropsychiatric conditions, especially schizophrenia.

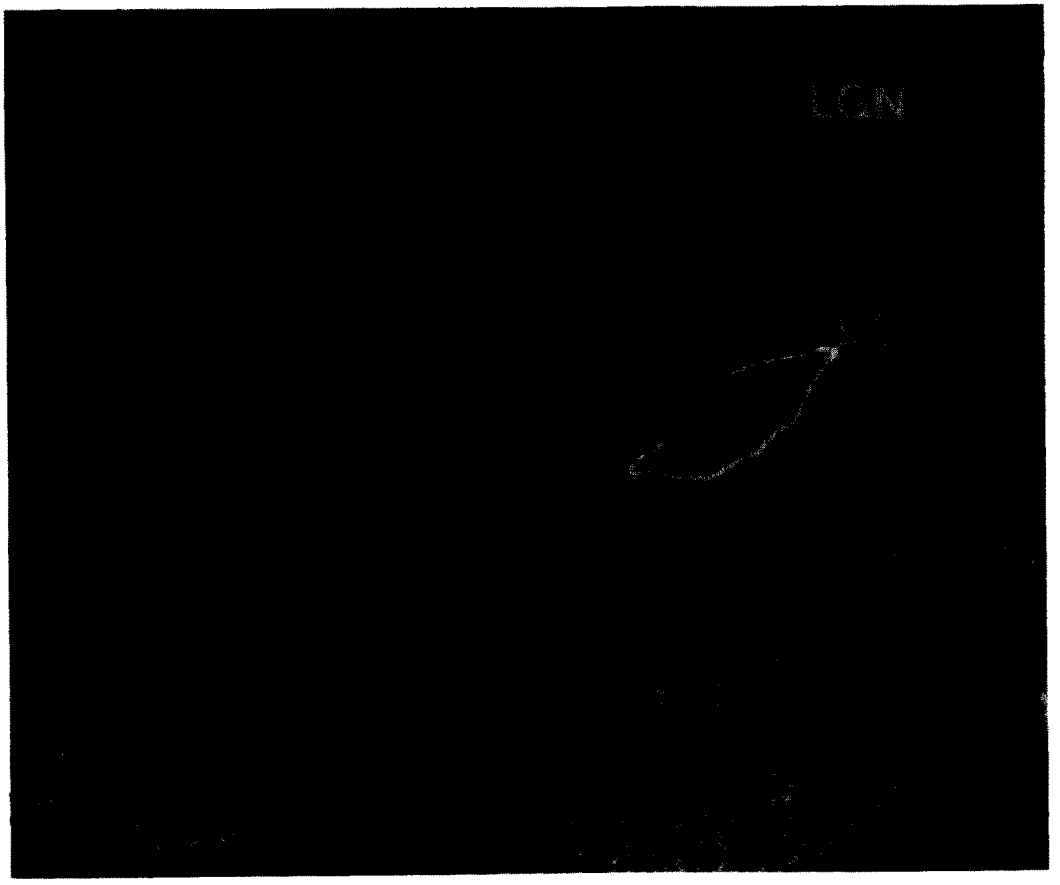


Fig 4. $D_2$ dopamine receptor mRNA in the hippocampus. Note striking labelling in the dentate gyrus (DG), as well as distinct lamination of labelling in the hippocampus as well as in the cortex. The lateral geniculate nucleus (LGN) is also visible

## Conclusion

Dopamine D2receptor mRNA was visualized in a number of areas of the monkey brain, including both motor and limbic terminal fields, as well as in the substantia nigra. It is likely that both postsynaptic receptors as well as autoreceptors are being visualized. The distribution of this mRNA is strikingly similar to what we and others have observed and reported in the rat brain. This report demonstrates the feasibility of visualizing dopamine $D_2$ receptor mRNA in the primate brain, and will hopefully set the stage for the subsequent investigation of the expression of the $D_2$ receptor gene in human brain in a variety of neuropsychiatric diseases.

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