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Excitant activity of methyl derivatives of quinolinic acid on rat cortical neurones

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1 Various synthetic analogues of quinolinic acid have been tested for agonist and antagonist properties when applied by microiontophoresis to neurones in the rat cerebral cortex.

2 Quinolinic acid 2-methylester was a weak antagonist of N-methyl-D-aspartate (NMDA) and quinolinic acid, but also showed agonist activity, being about half as active as quinolinic acid. The excitant effects of the compound could be antagonized by the NMDA receptor blocker, 2-amino-7-phosphonoheptanoic acid (2APH). N-methyl-quinolinic acid 2, 3-dimethylester showed very weak agonist and antagonist activity.

3 Homoquinolinic acid was a potent excitant of cortical neurones, being about five times more active than quinolinic acid and approximately equipotent with NMDA. The excitations were blocked by 2APH or its pentanoate analogue (2APV). Homoquinolinic acid 2-methylester was also active as an agonist.

4 N-methyl-DL-glutamic acid was also tested, since homoquinolinic acid is a rigid analogue of this compound. Although it did cause excitation of 5 of the 16 units tested, N-methyl-glutamate was a weaker agonist than NMDA or homoquinolinate.

5 Phthallic acid, ejected as an anion caused excitation of 14 out of 16 units. It is therefore concluded that the ring nitrogen of quinolinic acid is not essential for excitant activity.

6 Since homoquinolinic acid is a rigid analogue of glutamic acid, but causes excitation by acting apparently on the NMDA receptor, the results are consistent with the suggestion that activation of the NMDA receptor may require the carboxyl groups to be held in a relatively extended configuration.

Introduction

We have previously shown that quinolinic acid (pyridine-2, 3-dicarboxylic acid) is excitatory when applied to neurones in the rat cerebral cortex, being approximately as active as L-glutamate or L-aspartate (Stone & Perkins, 1981; Perkins & Stone 1983a, b). In other species quinolinic acid may be even more active, as Herrling, Morris & Salt (1983) have shown it to be half as active as N-methyl-D-aspartate in the cat caudate nucleus. As quinolinic acid is known to exist naturally in the mammal (Nishizuka & Hayaishi, 1963; Gholson, Ueda, Ogasawara & Henderson, 1964) and there are marked differences of neuronal sensitivity throughout the neuraxis (Perkins & Stone, 1983a, b) it is possible that quinolinic acid may play a physiological role in determining the excitability of neurones bearing amino acid receptors (Curtis & Johnston, 1974; Watkins & Evans, 1981).

A second reason why quinolinic acid is of interest is that it appears able to activate selectively the population of neuronal receptors for which N-methyl-D-aspartate (NMDA) is the preferred agonist (Watkins & Evans, 1981) as its excitant activity can be blocked by 2-amino-5-phosphono-valeric acid (Stone & Perkins, 1981) or 2-amino-7-phosphono-heptanoic acid (Perkins & Stone, 1983b). Yet although quinolinate is an analogue of NMDA, it is a rigidly planar molecule, by virtue of the aromatic ring, whereas NMDA is a non-planar flexible molecule. Quinolinic acid and derivatives may therefore yield valuable information about the structural requirements of the NMDA receptor and we have now begun a study of structure-activity relationships.

Methods

Male rats (sixteen) were anaesthetized with urethane, 1.5 g kg^{-1} i.p. and held in a stereotaxic frame. The cerebral cortex was exposed and the dura

mater removed. The exposed surface of the cortex was covered with warmed 0.9% w/v NaCl solution (saline) throughout the experiment and the rectal temperature was maintained at 37–38°C by an automatically regulated heating blanket.

All drugs were applied by microiontophoresis from seven-barrelled micropipettes containing glass fibres to permit rapid filling of the barrels immediately before use. The tip of the micropipettes was broken back to an overall size of 4–8 μm under microscopic observation. A separate single glass microelectrode containing 1 M potassium acetate (d.c. resistance 5–15 MΩ) was glued alongside the multibarrel assembly for recording unit activity (Stone, 1973). The tip of the single electrode was arranged to protrude no more than 5 μm beyond the multibarrel. Spikes were amplified, gated by a window discriminator, counted and displayed on a Grass polygraph either by a resetting integrator (reset time 1 s) or by a continuous ratemeter with a time constant of 2 s. When testing the effects of compounds as antagonists, agonists were applied in an automatically controlled time cycle so as to reduce any variations of response size due to retaining and ejecting currents (Bradshaw, Roberts & Szabadi, 1973).

Two methods of quantifying responses are frequently used. One is to calculate the amounts of compound ejected in terms of the electrical charge passed (current × time) to produce comparable peak responses, and the second is to determine only the current required to produce plateau responses of similar size. The latter method has been used here as it allows comparison to be made under equilibrium conditions. It should not be forgotten however, that such an equilibrium will depend on many factors including diffusion, the spacing of receptor populations, uptake processes, metabolism etc. It will also depend on the transport numbers of the compounds, which could not be determined in the present study due to the unavailability of radiolabelled material. In assessing relative potency, a series of responses were obtained in which the ejecting currents were adjusted to produce plateau firing rates as similar as possible. The ratio of ejecting currents was then used as the measurement of relative potency.

As electrodes can be extremely variable in the amount of material ejected at least five different electrodes were used in the calculation of a potency ratio.

The iontophoretic barrels were filled immediately before use with a selection of the following: N-methyl-D-aspartic acid (NMDA) 10 mM, pH6; quisqualic acid, 10 mM, pH7 (Cambridge Research Biochemicals); quinolinic acid, 20 mM pH 7; L-glutamate sodium, 50 mM, pH 7 (Sigma); N-methyl-DL-glutamic acid 20 mM pH 7 (Sigma); (±)-2-amino-7-phosphono-heptanoic acid (2APH) 10 mM, pH 7; phthallic acid, 25 mM, pH 8; (±)-2-amino-5-phosphono-valeric acid, (2APV) 10 mM, pH 7; 2-carboxypyridine-3-acetic acid (homoquinolinic acid) 10 mM, pH 7; homoquinolinic acid 2-methylester (H2ME) 20 mM, pH 7; quinolinic acid 2-methylester (QA2ME) 20 mH, pH 6; N-methyl-quinolinic acid 2, 3-dimethylester (MQDME) 10 mM, pH 3.5. All compounds except the last were ejected anionically, and pH was adjusted where necessary with 1 M NaOH or 1 M HCl. Homoquinolinic acid, QA2ME, MQDME, H2ME and 2APV were synthesized by Dr J.F. Collins (London) and 2APH was synthesised by Dr K. Curry (Vancouver).

One barrel of the multibarrel pipette was filled with 1 M NaCl at pH 6 for current balancing and to enable testing of current effects. Results from any cell which exhibited changes of firing in response to

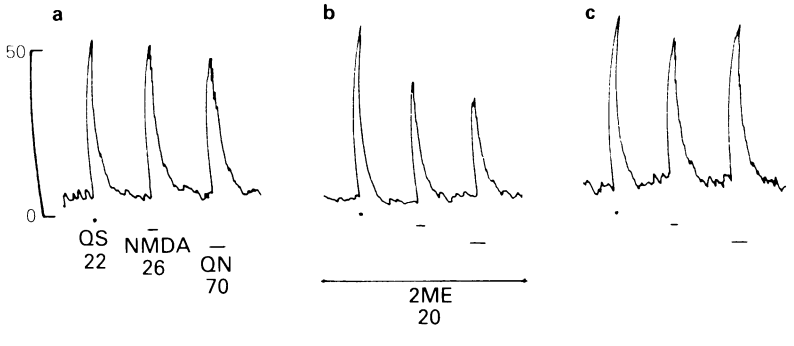

Figure 1 Records of the firing rate of a cortical neurone in response to the iontophoretic application of quisqualic acid (QS), NMDA, and quinolinic acid (QN). (a) Recorded before ejecting quinolinic acid 2-methylester (2ME) and (b) was recorded 4 min after beginning the 2ME ejection. (c) Shows recovery 5 minutes later. The number accompanying the drug abbreviation in this and subsequent figures indicates the ejecting current in nA. The ordinate scale is of firing rate in spikes per second and the time bar is 1 minute.

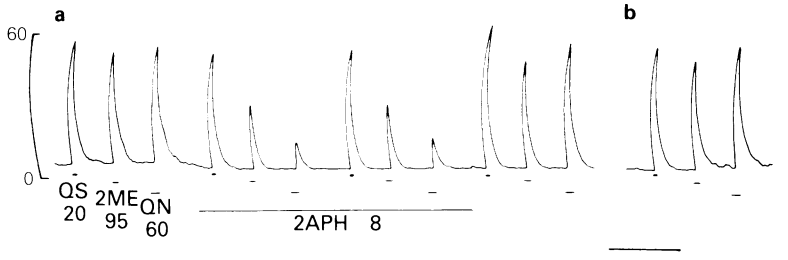

Figure 2 Record of the firing rate of a cortical neurone excited by the iontophoresis of quisqualic acid (QS), quinolinic acid 2-methylester (2ME) and quinolinic acid (QN). The application of 2APH reduces the responses to QN and 2ME without affecting QS. Details as for Figure 1.

control currents were discarded. All recordings were made at a depth of 1,000 to 2,000 μm in the somatosensory region of the cerebral cortex. This area is normally centred around a point 6 mm lateral to the midline and 1 mm anterior to the bregma suture.

Results

Quinolinic acid 2-methylester (QA2ME)

QA2ME was tested as an antagonist against a combination of quisqualate, quinolinate and NMDA on a total of 26 cells. Quisqualate excitations were never affected, though some evidence of a reduction of responses to NMDA and quinolinate was seen on 10 cells when QA2ME was ejected with low currents of 30 nA or less for several minutes (Figure 1). The reduction of NMDA peak response size on these 10 cells only was $26 \pm 9\%$ (s.e.mean, $n = 10$). On all these cells responses to NMDA and quinolinate were reduced approximately in parallel with little consistent evidence that QA2ME could distinguish between them.

However, QA2ME induced an increase of baseline firing of 16 of the 26 cells. At ejecting currents of 50–100 nA QA2ME was able to evoke a peak of activity comparable in profile with the other excitants used. Comparing on individual cells the currents required to produce plateau responses of similar size, the current ratio QA2ME: quinolinate proved to be 2.11 ± 0.20 (s.e.mean, $n = 16$) while the ratio for QA2ME : NMDA was 9.60 ± 1.3(16). Thus QA2ME appears to be about half as active as quinolinate and one tenth as active as NMDA.

On 8 of the cells on which clear excitatory responses to QA2ME were obtained, 2APH was applied with currents of 5 to 25 nA. On all 8 cells, the responses to quinolinate and QA2ME were reduced by 2APH while quisqualate was unaffected (Figure 2). However, QA2ME was always more resistant to antagonism than quinolinate, an observation well illustrated by the cell in Figure 2.

N-methyl-quinolinic acid 2, 3-dimethylester (MQDME)

MQDME proved to have very weak agonist and antagonist properties. Applied alone to 16 cells using currents of up to 100 nA, MQDME caused a slowly developing increase of firing rate, over a period of about 2 min, on 5 of them. When tested as an antagonist, MQDME caused a reduction of up to 20% of the peak response to NMDA on only 4 of the 16 units.

Homoquinolinic acid

Tests with homoquinolinic acid (see Figure 3) were made on a total of 42 cortical neurones, and it was rapidly apparent that homoquinolinate was an agonist, not antagonist. On some of these neurones the ratio of ejecting currents required to produce responses of comparable size was calculated, using NMDA as a standard. Whenever possible plateau responses were obtained as illustrated in Figure 4 and the results are summarised in Table 1, from which it can be seen that homoquinolinic acid was about 5 times more potent as an excitant than quinolinic acid. The greater potency of homoquinolinic acid was a consistent finding observed with 12 different electrodes. Indeed it was more potent than NMDA on a

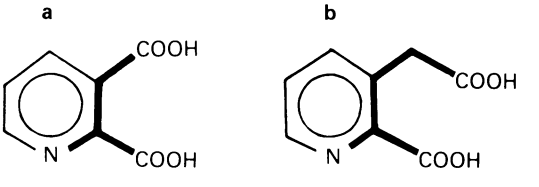

Figure 3 The structural formulae of (a) quinolinic acid and (b) homoquinolinic acid. The parts indicated by heavy lines are the carbon skeletons of aspartic and glutamic acids respectively, though it should be noted that these aliphatic acids are saturated and their 3-dimensional structure will not therefore be identical with the groups indicated.

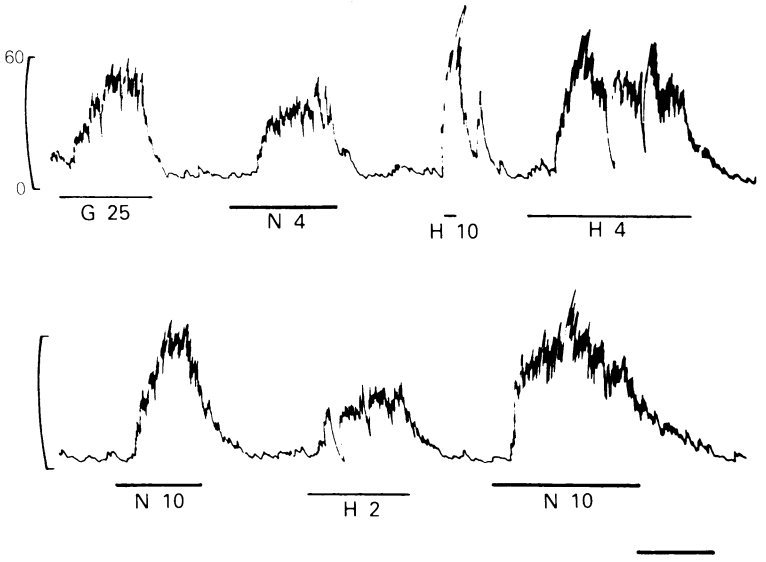

Figure 4 Record of the firing rate of a cortical neurone showing plateau responses to L-glutamate (G), NMDA (N), and homoquinolinic acid (H). The two parts of the record are continuous. Details as for Figure 1.

number of neurones (see Figure 4), although from the total sample of cells tested the homoquinolinate : NMDA current ratio was not significantly different from one (Table 1).

An attempt was made to define the type of receptor mediating homoquinolinate excitation using 2APH or 2APV. On 15 of 16 neurones tested, one or other of these antagonists blocked responses to both NMDA and homoquinolinate without affecting quisqualate (Figure 5). On most of these cells there was a suggestion that homoquinolinate was slightly more resistant than NMDA to antagonism by the phosphonates, but at the time of total NMDA blockade, homoquinolinate responses were always reduced by 75 to 95% (mean 88%).

Table 1 Ratios of agonist ejecting currents required to produce plateau responses of similar amplitude, relative to N-methyl-D-aspartate (NMDA)

	*Current ratios (compound : NMDA)**
NMDA	1.0
Glutamic acid	3.8 ±0.6 (12)**
Quinolinic acid	4.0 ±0.9 (7)
Quisqualic acid	0.22 ± 0.06 (16)
Homoquinolinic acid	0.90 ± 0.05 (25)

*The lower the ratio the more potent the compound

**Arithmetic mean ± s.e.mean (n).

Homoquinolinic acid 2-methylester (H2ME)

H2ME also proved to have very marked agonist activity which precluded any attempt to detect antagonist properties. When applied to 24 neurones H2ME caused excitation of 18, with ejection currents comparable with those used for homoquinolinate (Figure 6).

On 6 out of 9 neurones tested the iontophoresis of 2APH caused a parallel blockade of responses to NMDA and H2ME without reducing responses to

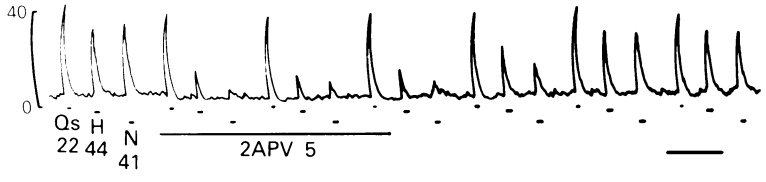

Figure 5 Record of the firing rate of a cortical neurone in response to the iontophoresis of quisqualic acid (QS), homoquinolinic acid (H) and NMDA(N). The application of 2APV greatly reduced responses to H and N with no discernible effect on QS. Details as for Figure 1.

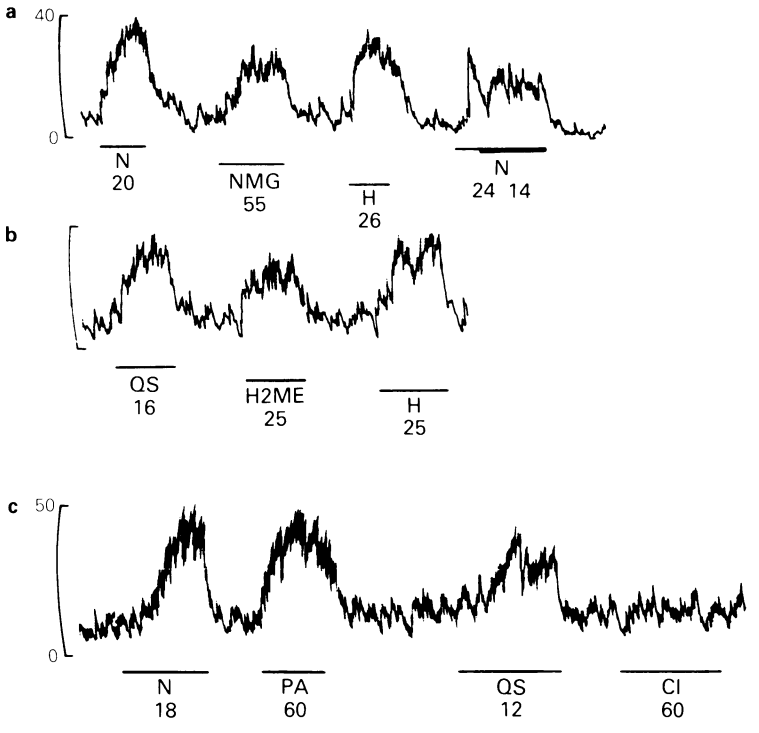

Figure 6 Records of the firing rate of cortical neurones showing plateau responses to NMDA (N), N-methyl-glutamate (NMG), homoquinolinic acid (H), quisqualic acid (QS), homoquinolinic acid 2-methylester (H2ME) and phthallic acid (PA). The lack of effect of anionic current is shown by the passage of 60 nA through the NaCl containing barrel (Cl 60). Traces (a) and (b) are consecutive recordings from the same neurone, trace (c) is taken from a different cell. Details as for Figure 1.

quisqualate (Figure 7). On the remaining 3 cells all three agonist were reduced in parallel.

N-methyl-glutamic acid

N-methyl-glutamate was applied to 16 cortical neurones with currents of up to 80 nA for 60 s. Five of these cells were excited by the application, though the responses were invariably weaker than those to NMDA or homoquinolinic acid. A current of 6 to 10 times that needed for a comparable response to NMDA was usually required, though on the most responsive of these cells a current only 4 times greater was needed (Figure 6). N-methyl-glutamate was also tested as a possible antagonist of NMDA (13 cells) or quinolinate (9 cells) when applied for up to 4 min with currents of 40 nA. No change of responses to either agonist could be detected.

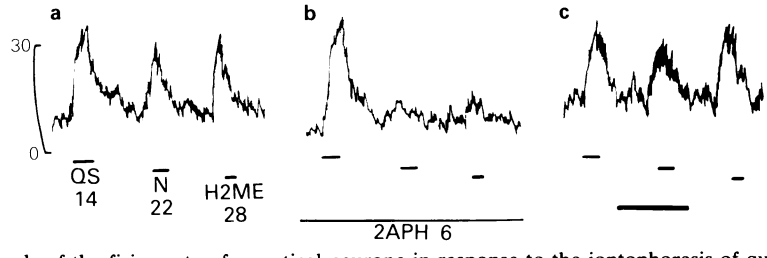

Figure 7 Records of the firing rate of a cortical neurone in response to the iontophoresis of quisqualate (QS), NMDA (N) and homoquinolinic acid 2-methylester (H2ME). The application of 2APH for 3 min before and during (b) greatly reduced responses to N and H2ME without affecting QS. Trace (c) was recorded 6 min after ending the 2APH ejection. Details as for Figure 1.

Phthallic acid

Phthallic acid was ejected as an anion on to 16 cells. At currents of 40–75 nA for 10 to 30 s, it caused excitation of 14 of these (Figure 6), the responses being comparable in magnitude to those produced by quinolinic acid at comparable doses.

Discussion

It must be emphasized that the calculations and comments on potency in this paper are subject to the technical limitations imposed by the iontophoretic technique. This includes uncertainty as to the precise site of application of compounds and a lack of information on the concentrations achieved by ejection. The latter problem could be partly resolved by the determination of transport numbers, but the compounds used here are not available in radiolabelled form at present. It could be argued that a more meaningful experimental protocol would be to compare potencies in different brain regions, as has been achieved for quinolinic acid (Perkins & Stone, 1983a, b). This will probably be attempted at least for homoquinolinic acid, in the near future. Nevertheless while the esters of quinolinic and homoquinolinic acids have less polar molecules, which may not be ejected as well as the parent acids, the structures of quinolinic acid and homoquinolinic acid themselves are sufficiently similar that a major difference of transport number would not be predicted and almost certainly cannot account for the consistent finding of a 5 fold difference of activity between these compounds.

Clearly within these limitations the methylesters of quinolinic acid are unlikely to prove useful as antagonists of NMDA or quinolinic acid when applied by microiontophoresis. Indeed the esters themselves showed excitant activity, suggesting that both carboxyl groups need not be intact for excitatory activity. The relatively high activity of QA2ME compared to quinolinate (about half) strongly suggests that the activity of the ester is not due merely to hydrolysis in solution. This is also supported by the greater susceptibility of quinolinate compared to QA2ME, to antagonism by 2APH.

The explanation for this relative resistance of QA2ME to antagonism by 2APH is not yet clear. The existence of another receptor is one possible explanation. It is also conceivable that the differential antagonism is due to a different ability of the two compounds to diffuse to distant receptors. Thus QA2ME could diffuse to receptors located on parts of the neurone which other compounds cannot reach (e.g. 2APH), whereas quinolinic acid itself may not.

Of greatest interest, however, is the observation that homoquinolinic acid is as potent an excitant as NMDA, being about five times more active than quinolinic acid. The original motivation for testing homoquinolinate was the possibility that it might prove to be an antagonist, partly on the grounds that homoquinolinate may be considered to be a rigid analogue of N-methyl-glutamate (Figure 3) which Curtis & Watkins (1960) reported to have no agonist effect on spinal neurones. It appears from the present study that N-methyl-glutamate can cause some excitation of cortical neurones, a finding consistent with the suggestion that pharmacological differences may exist between spinal cord and cortical aminoacid receptors (Perkins & Stone, 1983a). However, the excitation is too weak, and observed too infrequently, to account for the activity of homoquinolinate in terms of a cortical N-methyl-glutamate receptor.

However, in spite of its structural similarity to glutamate, homoquinolinate excitations could be antagonized by 2APV or 2APH, compounds which discriminate well between the NMDA and quisqualate receptors on cortical neurones at the doses used here (Figures 5 and 7; Perkins, Stone, Collins & Curry, 1981; Stone, Perkins, Collins & Curry, 1981). This implies that homoquinolinate is acting largely via the NMDA site and thus supports the view that activation of the NMDA receptor depends primarily on the presence of two carboxyl groups held in an extended conformation as proposed by McLennan, Hicks & Liu (1982), and is less dependent on whether the carbon skeleton is of aspartate or glutamate length.

A further observation of some interest was the excitant activity of phthallic acid. We have previously reported that this compound was inactive as an excitant (Birley, Collins, Perkins & Stone 1982), but this appears due to the fact that, in view of its low solubility, a weak solution of phthallic acid was made in acidified NaCl solution and ejection was attempted using an outward current. It has now been realized that phthallic acid is quite soluble but only slowly so, and its anionic ejection here from a solution of pH 8 clearly caused excitation comparable with quinolinic acid. This implies that the aromatic nitrogen of quinolinate is not important for excitatory activity. On the other hand non-aromatic dicarboxylic compounds lacking a nitrogen atom such as succinic acid or its unsaturated analogue maleic acid are not excitatory, at least in spinal cord (Curtis & Watkins, 1960). Furthermore in preliminary experiments we have found that homophthallic acid is far less excitatory than phthallic, quinolinic or homoquinolinic acids. Resolution of this paradox could add greatly to an appreciation of the molecular features necessary for activation of dicarboxylic acid receptors.

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