3.1. – ENVOLVIMENTO DOS RECEPTORES *N*-METIL-D-ASPARTATO NA ATIVIDADE PRÓ-OXIDANTE DO ÁCIDO QUINOLÍNICO, MAS NÃO NA ATIVIDADE PRÓ-OXIDANTE DO MALONATO *IN VITRO*

Artigo 1

N-METHYL-D-ASPARTATE RECEPTORS ARE INVOLVED IN THE QUINOLINIC ACID, BUT NOT IN THE MALONATE PRO-OXIDATIVE ACTIVITY IN VITRO

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N-methyl-D-aspartate Receptors are Involved in the Quinolinic Acid, but not in the Malonate Pro-oxidative Activity *in vitro*

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Oxidative stress plays a significant role in the neurotoxicity of a variety of agents that interact with the *N*-methyl-D-aspartate (NMDA) receptors. Here we investigated in a comparative way the pro-oxidative effects of quinolinic acid (QA) and malonate, two neurotoxic substances that act through distinct primary molecular mechanisms on the production of thiobarbituric acid reactive species (TBARS) by brain homogenates. In fact, QA is thought to activate directly the NMDA receptor, whereas malonate seems to act primarily by inhibiting oxidative metabolism. The malonate-induced TBARS formation was not modified by cyanide (CN^-) or 2,4-dinitrophenol. MK-801 did not reduce basal or malonate induced-TBARS production in fresh tissues preparations. However, in heat-treated preparations a significant effect of MK-801 against basal TBARS production was observed, but not on the malonate induced-TBARS production. QA induced-TBARS production was significantly prevented by MK-801 either in fresh or heat-treated preparations. The antioxidant effect of MK-801 on basal and QA-induced TBARS production increased as the temperatures used to treat S1 were increased. Succinate dehydrogenase (SDH) was inhibited by malonate but not by QA. Malonate was able to chelate iron(II) and the malonate-iron complex(es) is(are) active as measured by its(their) activity on deoxyribose degradation assay. These findings indicate that direct interactions of malonate with NMDA receptors are not involved in malonate pro-oxidative activity *in vitro*. QA pro-oxidative activity *in vitro* was related, at least in part, to its capability in stimulate NMDA receptors. Taken together, these findings indicated that malonate pro-oxidative activity *in vitro* could be attributed to its capability of changing the ratio Fe^{2+} / Fe^{3+}, which is essential to TBARS production.

KEY WORDS: Iron; malonate; NMDA receptors; pro-oxidant; quinolinic acid.

INTRODUCTION

Malonate is a reversible inhibitor of mitochondrial succinate dehydrogenase (SDH) and, consequently can induce mitochondrial dysfunction. In fact, malonate can trigger the generation of superoxide radicals, secondary excitotoxicity mediated by Ca^{2+} influx, and apoptosis (1). Several earlier studies have demonstrated that the neurotoxicity associated with intrastriatal injection of malonate is mediated almost exclusively by the indirect activation of *N*-methyl-D-aspartate (NMDA) receptors (2–5). The mechanism underlying malonate induced-neurotoxicity seems to involve ATP exhaustion, which causes depolarization. However, although MK-801 reduced the malonate-induced lesion volume in the striatum,

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it did not block the generation of reactive oxygen species (ROS), indicating that NMDA receptor activation occurs but does not result in the generation of ROS in malonate toxicity (6, 7). In contrast with this, other studies have shown that NMDA receptors activation could be involved in the observed increased in ROS levels (8, 9), thus contributing to oxidative stress due to malonate.

In the rat, acute (2, 4, 5, 10) and chronic (11) intrastriatal administration of malonate produces dose-dependent lesions with features similar to those produces by quinolinic acid (QA), an agonist of NMDA receptor (12). In line with this, malonate exacerbates the neurotoxicity of glutamate and NMDA (10, 13). Thus, although some discrepancies still exist in literature about the involvement of ROS generation via NMDA activation on the neurotoxicity of malonate, a consensus has emerged in the literature connecting glutamate receptor activation with neuronal damage due to malonate (2, 13).

The mechanism underlying QA induced-neurotoxicity seems to involve direct activation of the NMDA receptor, either by pre-synaptically stimulating the release of the excitatory amino acid transmitter or by direct post-synaptic action, which in turn produces excitotoxicity (8, 14). In fact, it has been proposed that over stimulation of excitatory amino acid receptors is involved in the pathophysiology of acute brain injury and chronic neurodegenerative disease (15, 16) that can be associated, at least in part, with the over production of reactive oxygen and nitrogen species caused by glutamate, NMDA or QA (17–20).

Taking into account that *in vivo* and *in vitro* QA can exacerbate considerably the production of ROS and lipid peroxidation (17, 21, 22) and that lipid peroxidation is initiated by the attack of free radicals against unsaturated membrane lipids, it is reasonable assume that QA toxicity is mediated by ROS formed after NMDA receptors activation (8, 9). In addition, QA-induced lipid peroxidation is blocked by MK-801 and a variety of antioxidants (9, 22–28) giving further support for NMDA receptor activation and ROS overproduction on its toxicity. However, there are still controversies in the literature on whether NMDA receptors activation and/or ROS are involved in the neuronal cell death caused by malonate (6, 13).

Therefore, due to inconsistent data reported in the literature with respect to the involvement of NMDA receptors activation in the malonate neurotoxicity and ROS overproduction (6, 13, 29), the aim of this study was to investigate whether NMDA receptors are involved in the malonate and QA pro-oxidative activity measured *in vitro* by their ability in stimulating brain lipid peroxidation. In the present study, we used MK-801, to investigate the NMDA receptor involvement in the toxicity of these neurotoxic agents. Furthermore, the possible involvement of respiratory chain reactions on the pro-oxidative activity of these compounds was investigated by inactivating brain homogenates with heating.

MATERIAL AND METHODS

Tissue Preparation

Adult rats (inbreed Wistar strain) from our own breeding colony were maintained in an air-conditioned room (20–25°C) under natural lighting conditions (the luminosity of the colony room was determined by the environmental situation), with water and food (Guabi, Ribeirão Preto, SP, Brazil) *ad libitum*. Animals were anesthetized with ether and killed by decapitation. The brain was quickly removed, placed on ice, and homogenized within 10 min, in 10 volumes of cold 100 mmol/l NaCl. The homogenate was centrifuged at 4000 × *g* at 4°C for 10 min to yield a low speed supernatant fraction (S1) that was used immediately for thiobarbituric acid reactive species (TBARS) assay.

Lipid Peroxidation Assay

Lipid peroxidation was determined by measuring TBARS as described by Ohkawa et al. (30). Brain homogenates were prepared by homogenization as described above. Aliquots of the homogenate (100 µl) from brain were incubated at 37°C in a water bath for 60 min in a medium containing 10 mM Tris/HCl buffer, pH 7.4 in the presence of other reagents at concentrations indicated in each figure legend. The reaction was stopped by 0.5 ml of acetic acid buffer and lipid peroxidation products were measured by the addition of 0.5 ml of TBA 0.6% and 0.2 ml of SDS 8.1%. The color reaction was developed by incubating tubes in boiling water for 60 min. TBARS levels were measured at 532 nm using a standard curve of MDA. The values are expressed in (µmol MDA/g of tissue. Were indicated, the S1 fraction was pre-treated for 10 min at indicated temperature before the incubation at 37°C for 60 min.

SDH Activity Assay

SDH activity was estimated as described by (31). The reaction mixture containing 50 mmol/l potassium phosphate buffer (pH 7.4), 0.5 mmol/l succinate (pH 7.2), 10 mmol/l sodium azide, and 0.8 mmol/l INT was warmed for 1 min in a water bath at 37°C. Then 100 µl of the tissue (S1) was added to initiate the reaction. The total volume of the reaction mixture was 0.5 ml. After incubation for 10 min, 1.5 ml of 95% alcohol was added. After 15 min on ice, tubes were centrifuged for 10 min at 800 × *g* at room temperature. The absorbance of 1 ml of clear alcohol-H_2O extract was read at 458 nm (formazan formatted) in a spectrophotometer, the zero-reference cuvette contained H_2O. Basal reduction of INT was determined in control tubes in which

succinate was omitted from the reaction mixture, and this value was subtracted from the absorbance that was measured for the experimental tube. Where indicated, S1 was pre-treated at 100°C for 10 min and, after SDH activity was assayed.

Chelating Properties of the Malonate Assay

To examine iron chelating properties of malonate, we used the *o*-phenantroline method as previously described (32, 33). Iron (II) was added to the buffered medium containing 25 mmol/l NaCl and 10 mmol/l Tris/HCl buffer, the mixture containing Fe^{2+} 150 mol/l and malonate at indicated concentrations was allowed approximately by 5 min to complex(es) formation between iron(II) and malonate, than *o*-phenantroline solution was added to determine the presence of the colored complex(es) formed between *o*-phenantroline and free iron (II). A 0.25% *o*-phenantroline solution in ethanol was used in the analyses. Absorbance values were proportional to the amount of free Fe^{2+}; therefore a decrease in absorbance indicates that the malonate exhibited chelating properties.

The absorbance was recorded at 510 nm. The values are expressed in percentage of control determined in the absence of malonate. Solutions of $FeSO_4$ were made just before use in distilled water.

Degradation of Deoxyribose Assay

Reactions mixtures contained, in a final volume of 0.8 ml, the following reagents at the final concentrations stated: deoxyribose 3 mmol/l, KH_2PO_4 buffer 50 mmol/l, pH 7.4, $FeSO_4$ 50 µmol/l and H_2O_2 500 µmol/l. Solutions of $FeSO_4$ and H_2O_2 were made just before use in distilled water. Reaction mixtures were incubated at 37°C for 30 min and stopped by the addition of 0.8 ml of TCA 2.8%, followed by the addition of 0.4 ml of TBA 0.6% solution. Tubes were incubated in boiling water for 20 min and then the absorbance was recorded at 532 (34, 35). Standard curves of MDA were made in each experiment. The values are expressed in (µmol MDA/l.

Statistical Analysis

Data were analyzed by one-way ANOVA, followed by Tukey Multiple Range Test when appropriate. Differences between groups were considered to be significant when $P \leq 0.05$.

RESULTS

Effect of Malonate on Basal-TBARS Production

Malonate induced a concentration dependent increase in the TBARS production (Fig. 1), which was significant from 1 mmol/l onwards. The malonate effect was not modified by the addition of potassium cyanide (KCN) 1 mmol/l (Fig. 1). Comparisons between groups of the curve obtained in the absence of KCN with that of groups of the curve obtained in the presence of KCN were done by one-way ANOVA followed by Tukey multiple range test.

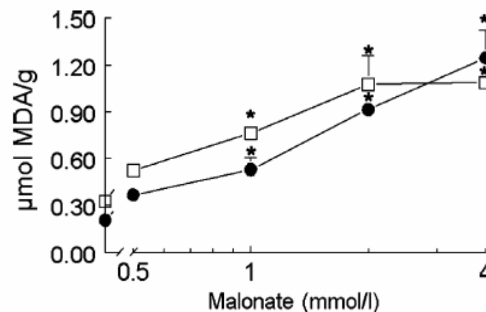

Fig. 1. Effect of malonate on basal-TBARS production. Low-speed supernatant (S1) from brain was incubated for 60 min in a medium containing 10 mmol/l Tris/HCl buffer, pH 7.4, in the presence of the indicated concentrations of malonate. TBARS are expressed as (µmol of MDA per g of tissue. —●— no addition, —□— KCN 1 mmol/l. The determinations were done in the absence of any other Krebs cycle substrate. Data are expressed as means ± SEM (n = 3). One-way ANOVA followed by Tukey multiple range test revealed no significant differences between the groups in the curve with cyanide, when compared to the groups in the curve without cyanide. *Indicate a $P < 0.05$ from respective control by Tukey multiple range test.

Statistical analysis revealed no significant difference between KCN groups and non-cyanide groups. 2,4 dinitrophenol (0.1–10 µmol/l) did not modify basal or malonate (4 mmol/l) induced-TBARS production (data not shown).

Effect of Succinate on Basal or Malonate–induced TBARS Production

Succinate (8 mmol/l), a substrate of SDH, attenuated the malonate (4 mmol/l) induced increase in TBARS production (Fig. 2). However, succinate did not revert malonate-induced TBARS to basal values and succinate reduced significantly the basal-TBARS production.

Effect of MK-801 on Basal, Malonate or QA-induced TBARS Production

In order to investigate the involvement of NMDA receptors in the malonate pro-oxidative activity, we measured malonate induced-TBARS production in the presence or absence of the MK-801 in fresh- and heat-treated brain S1 (at 100°C for 10 min). Malonate (4 mmol/l) caused a marked increase in TBARS production either in fresh (444.7% compared to fresh basal values) or heat-treated (197.5% compared to heat-treated basal values) preparations (Table I). These results support the conclusion that malonate effect was at least in part

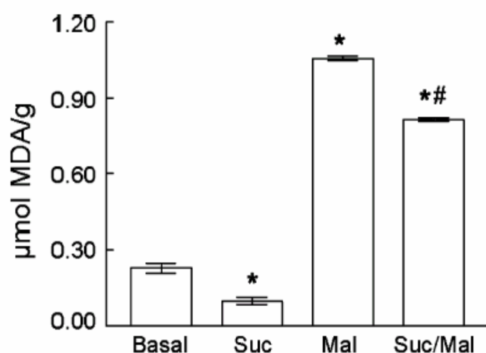

Fig. 2. Effect of succinate on basal or malonate–induced TBARS production. Antioxidant effect of succinate against basal or malonate induced TBARS production. The values are expressed as µmol of MDA per gram of tissue. Abbreviations: Suc: succinate (8 mmol/l); Mal: malonate (4 mmol/l). Data are expressed as means ± SEM (n = 3). $^{*}P < 0.05$ from basal and $^{\#}P < 0.05$ from malonate by Tukey multiple range test.

independent of respiratory chain reactions because this pre-treatment is expected to cause complete enzymatic inactivation. Accordingly, SDH an enzyme of Krebs cycle and component of respiratory chain was completely inactivated by this treatment (Fig. 3a). Furthermore, we also assayed lactate dehydrogenase of 100°C-treated S1 and its activity was completely inhibited by heat treatment (data not shown). The addition of MK-801 (1 mmol/l) was ineffective in prevent basal or malonate induced-TBARS production in fresh preparations (Table I). However, when we used heat-treated preparations, MK-801 significantly prevented basal TBARS production without abolishing malonate-induced TBARS production (Table I). These results suggested that NMDA receptors activation is not involved in the malonate induced TBARS production, and that oxidative stress due to malonate is not a downstream consequence of a secondary excitotoxicity in our *in vitro* conditions.

QA (1 mmol/l) caused a significantly increase in TBARS production either in fresh (62.5% compared to basal values) or heat-treated (35.4% compared to basal values) preparations (Table II). MK-801 (1 mmol/l) was ineffective against basal TBARS production in fresh preparations; however, exhibited a significant effect against basal-TBARS production in heat-treated preparations (Table II). MK-801 significantly reduced QA-induced TBARS production, either in fresh or heat-treated preparations (Table II). These results confirm the involvement of NMDA receptors activation in the QA pro-oxidative activity *in vitro*.

Effect of MK-801 on Basal or QA-induced TBARS Production in S1 Pre-treated at Different Temperatures

Exposure of S1 for 10 min to increasing temperatures caused an increase in basal-TBARS production (Table III) and a proportional decrease of QA-induced TBARS production. Note that at higher temperatures the QA-induced TBARS production was lower than that observed at lower temperatures (compare the 22.9% increase at 100°C with 41.3% at lower temperatures).

However, in the S1 treated at 100°C the reduction on basal TBARS caused by MK-801 was proportionally higher (56.1% of reduction) than that observed in S1 preparations pre-treated at lower temperatures (approximately 20%) (Table III). Similarly, MK-801 antagonizes more efficiently the QA-induced TBARS production in S1 pre-treated at 100°C than in those S1 preparations pre-treated at lower temperatures. After 10 min of pre-treatment of S1 at 100°C, the TBARS production in the presence of MK-801 plus QA was even lower than that of basal value (Table III).

Effect of Malonate and QA on SDH Activity

Malonate cause a significant inhibition of brain SDH (Fig. 3a) and the inhibition was significant from 0.1 mmol/l onwards.

QA has two carboxylic groups separated apart by about 0.2–0.4 nm, which is somewhat similar to

Table I. Effect of MK-801 on Basal or Malonate Induced-TBARS Production Either in Fresh or Brain S1 Preparations Treated at 100°C for 10 min

	Fresh	Heat-treated
Basal	212.33 ± 2.91 (100%)	499.47 ± 5.01[a](100%)
Malonate	1156.72 ± 196.44* (544%)	1486.05 ± 164.82* (297%)
MK-801	201.43 ± 2.15 (94%)	265.56 ± 52.32* (53%)
Malonate + MK-801	1117.81 ± 185.32* (526%)	1436.73 ± 213.89* (287%)

Data are expressed as mean ± S. E. (n = 3). Values are expressed as nmol MDA/g.
*From respective basal; [a]Different of respective control fresh by one way ANOVA following by Tukey test.

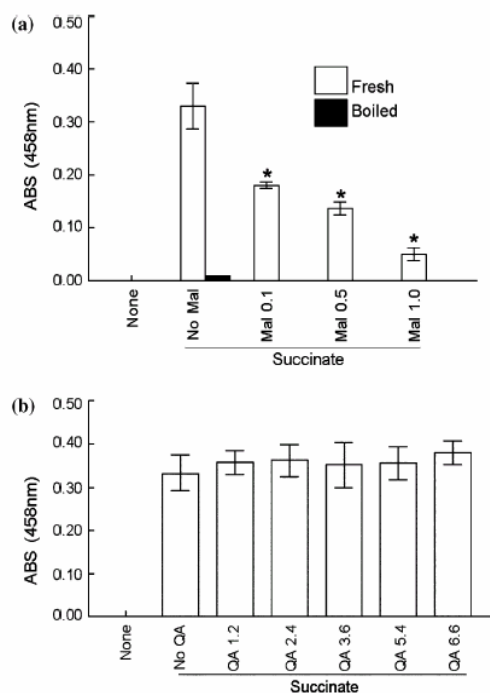

Fig. 3. Malonate and QA effect on the SDH activity. (a) Effect of malonate at indicated concentrations (mmol/l) on SDH activity. Values represent the difference between ABS (458 nm) without succinate and ABS with succinate (0.5 mmol/l). None: without succinate. Were indicated, S1 was pre-treated for 10 min at 100°C before SDH activity assay. Data are expressed as means ± SEM (n = 3). *P < 0.05 from control without malonate by Tukey multiple range test. (b) Effect of QA at indicated concentrations (mmol/l) on SDH activity. Values represent the difference between ABS (458 nm) without succinate and ABS with succinate (0.5 mmol/l). None: without succinate. Data are expressed as means ± SEM (n = 4).

that for other dicarboxylic acids, such as malonate, that are inhibitors of SDH. So, we tested whether QA could also inhibit SDH. However, QA (up to 6.6 mmol/l) did not inhibit SDH (Fig. 3b). Pre-treatment of S1 by heating at 100°C for 10 min completely inhibited SDH activity (Fig. 3a).

Table II. Effect of MK-801 on Basal or QA Induced-TBARS Production either in Fresh or Brain S1 Preparations Treated at 100°C for 10 min

	Fresh	Heat-treated
Basal	215.63 ± 2.67 (100%)	425.37 ± 20.29[a] (100%)
QA	350.43 ± 4.94* (162%)	575.95 ± 23.76*[a] (135%)
MK-801	170.81 ± 4.94 (79%)	196.12 ± 19.65* (46%)
QA+MK-801	274.06 ± 7.86*[b] (127%)	418.01 ± 27.65 [ab] (98%)

Data are expressed as mean ± S. E. (n = 3). Values are expressed as nmol MDA/g.
*From respective basal; [a]Different of respective control fresh; [b]Different of respective QA by one way ANOVA following by Tukey test.

Chelating Properties of Malonate

Literature data indicates that iron ions (particularly the ratio Fe^{2+}/Fe^{3+}) are an important factor that regulates lipid peroxidation *in vitro* (36, 37). Furthermore, the formation of complexes of iron with negative charged compounds can enhance the ability of iron to produce oxidative stress, possibly by changing the ratio Fe^{2+}/Fe^{3+}. So, we investigate whether malonate could chelate iron (Fig. 4) and whether the complex(es) formed with Fe^{2+} could enhance its pro-oxidative activities by measuring deoxyribose degradation (Fig. 5), a well-established model for studying iron driven Fenton reaction (34, 35). Malonate chelated Fe(II) in a concentration-dependent manner and this was significant from 4 mmol/l onwards (Fig. 4). The maximum reduction in free Fe^{+2} in the medium reaction (65%) was obtained at 32 mmol/l of malonate.

Effect of Malonate on Deoxyribose Degradation

Fe(II) or H_2O_2 stimulated deoxyribose degradation. However, in the presence of iron and H_2O_2 deoxyribose degradation was higher than that determined in the presence of compounds separately. Malonate did not modify the stimulatory effect of iron on deoxyribose degradation.

DISCUSSION

The results presented in this paper suggest that the pro-oxidative activity of malonate *in vitro* was at least in part independent of mitochondrial respiratory chain reaction(s), because heat treatment (which inactivated completely SDH and lactate dehydrogenase activity) did not abolish malonate effect. Furthermore, KCN and 2,4-dinitrophenol did not change malonate-induced TBARS production (data not shown).

In contrast to the malonate effect, the protective effect of succinate (Fig. 2) against either basal or malonate-induced TBARS production supports a role for SDH activity on antioxidant properties of succinate. However, the succinate-induced decreases in the basal and malonate groups were of similar magnitude. These suggest that the entire decline was due to a decline in the basal TBARS production. We hypothesized that succinate antioxidant activity under our conditions could be attributed at least in part to ubiquinol (38). In line with this, ubiquinol has

Table III. Effect of MK-801 on Basal or QA-Induced TBARS Production in S1 Treated at different Temperatures for 10 min

	0°C	25°C	37°C	50°C	100°C
Basal	214.96 ± 3.61 (100%)	222.85 ± 6.83 (100%)	217.77 ± 7.19 (100%)	300.32 ± 10.39 (100%)	409.75 ± 14.95 (100%)
QA	303.63 ± 4.55* (141%)	323.47 ± 7.93* (145%)	329.25 ± 17.30* (151%)	387.96 ± 5.49* (129%)	503.62 ± 32.48* (122%)
MK-801	173.67 ± 2.80 (80%)	185.16 ± 8.57 (83%)	182.45 ± 7.83 (83%)	243.23 ± 10.15* (80%)	180.08 ± 16.83* (43%)
QA + MK-801	266.76 ± 10.21*[a] (124%)	271.17 ± 13.05*[a](121%)	275.26 ± 16.03 (126%)	318.42 ± 11.33[a] (106%)	330.99 ± 45.12[a] (80%)

Data are expressed as mean ± S. E. ($n = 4$). Values are expressed as nmol MDA/g.
* from respective basal; [a] different of respective QA by Tukey test.

potent antioxidant activity in biological systems (39, 40).

The absence of a protective effect of MK-801 against malonate pro-oxidative activity may indicate that secondary excitotoxicity is not involved in the effect of malonate in brain homogenates. These results are in accordance with previous study showing that NMDA receptor over-stimulation does not contribute significantly to the oxidative stress in cultured cells exposed to malonate (7). The malonate-induced TBARS production under conditions in which biological activity has been inactivated by heat treatment were unexpected. In fact, one could expect that heat treatment could cause non-specific increase in TBARS production by accelerating the oxidation of polyunsaturated fatty acids from brain lipids. However, the increase was specific for some treatments (for instance, malonate and QA) and reversed by MK-801. The effect of malonate even when all of the enzymes have been inactivated suggests that malonate pro-oxidative activity can not be attribute exclusively to inhibition of SDH and possibly is related to its iron-chelating properties. In fact, heat-treatment could increase the release of iron from storage proteins, accelerating the ROS generation, and consequently TBARS production.

Based on iron-chelating malonate properties, we hypothesized that malonate pro-oxidative activity *in vitro* can be attributed to its interaction with endogenous free iron present in the medium of preparation. These complexes, such as iron-citrate (41), are active in our system, thus increasing free radicals generations due to redox cycle of iron. However, malonate did not change deoxyribose degradation caused by iron and H_2O_2, indicating that the iron-malonate complexes are active when a pure chemical system was used.

Our results confirm the hypothesis that QA pro-oxidative activity is mediated by a direct activation of NMDA receptors (8, 9), because MK-801 significantly prevented QA-induced TBARS production. How QA can work in heat-treated samples is not clear. It would appear that the heat treatment did not inactivate the glutamate receptors. The data obtained with heat-treated preparations indicate that heat-treatment activate NMDA receptor. In line with this,

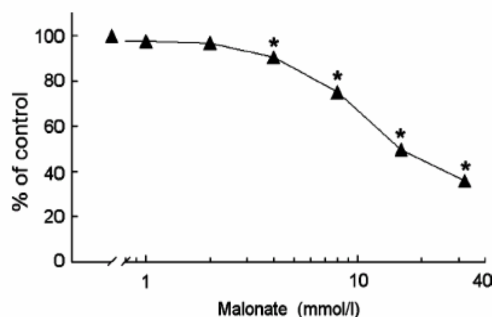

Fig. 4. Chelating property of malonate. Effect of malonate on colored iron-phenantroline complex formation. The values are expressed as percentage of control. Absorbance obtained by reaction between free Fe^{2+} with *o*-phenantroline in the absence of malonate is considered 100%. Data are expressed as means ± SEM ($n = 3$). *$P < 0.05$ from respective control by Tukey multiple range test.

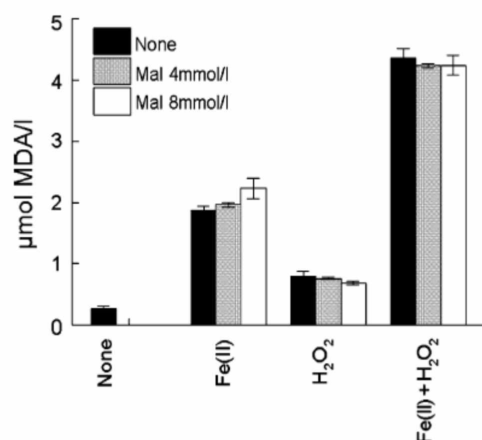

Fig. 5. Effect of malonate on deoxyribose degradation. Effect of malonate against deoxyribose degradation induced by Fe^{2+} 50 μmol/l and/or hydrogen peroxide 500 μmol/l. The values are expressed as (μmol MDA per liter. Data are expressed as means ± SEM ($n = 3$).

MK-801 was more efficient against either basal or QA-induced TBARS production when samples were exposed to increasing temperatures in comparison to fresh samples (see Table III). However, we did not exclude the potential intrinsic oxidative properties of QA (17), which can be related, at least in part with iron ions (42). These could partially explain how QA work in heat-treated samples. In spite of this, the protection afforded by MK-801 strongly suggests that part of its effect is mediated by NMDA receptor activation. These results are coincident with a previous study showing that the probability of the NMDA channel opening increases steeply with temperature up to near 50°C (43). To the best of our knowledge, there are no data on the literature about the effects of higher temperatures on NMDA activation. Furthermore, *in vivo* studies have demonstrated that hyperthermia aggravates seizures episodes, increasing brain damage mediated in part by NMDA receptors activation (44, 45). This result indicated that probably heat-treatment alters conformational structure of NMDA receptors leading to a permanent activation of NMDA receptor, which is prevented by MK-801. In line with these, the proportional decrease in the QA-induced TBARS production with temperature can be attributed to direct over activation of NMDA receptors by temperature.

In conclusion, our data suggest that malonate pro-oxidant activity *in vitro* is due to its ability to interact with endogenous iron, forming active complexes, thus contributing to maintenance of adequate ratio Fe^{2+}/Fe^{3+}. In addition, the results presented in this paper confirm the involvement NMDA receptors activation in the QA pro-oxidant activity *in vitro*. However, we cannot exclude that iron also modulates QA pro-oxidative activity *in vitro* by changing the ratio between Fe^{2+}/Fe^{3+} (42). In short, the effect of higher temperatures can involve an increase in the activation of NMDA receptor, inhibition of respiratory chain (ubiquinol formation), and release of iron from iron-binding proteins, whereas the pro-oxidative effect of QA involves NMDA receptor activation and the malonate pro-oxidative effect is mediated by a different mechanism that does not involves NMDA receptor.

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