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Antidepressant-like effect of diphenyl diselenide on rats exposed to malathion: Involvement of Na^+K^+ ATPase activity

Carmine I. Acker, Cristiane Luchese, Marina Prigol, Cristina W. Nogueira *

Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil

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ABSTRACT

The antidepressant-like effect of repeated administration of diphenyl diselenide $(PhSe)_2$ in rats exposed to malathion is reported. The role of Na^+K^+ ATPase, acetylcholinesterase (AChE) and monoamine oxidase (MAO) activities and oxidative stress in antidepressant behavior were investigated in cerebral cortex of rats. Rats were exposed once a day for 3 consecutive days to malathion (50 mg/kg, intraperitoneal) and $(PhSe)_2$ (50 mg/kg, oral). To investigate the antidepressant-like behavior rats were submitted to the forced swimming test (FST) and open-field test (OFT). Thiobarbituric acid reactive species (TBARS) levels, enzymatic and non-enzymatic antioxidant defenses were carried out in cerebral cortex of rats. The results confirmed that malathion increased immobility time in the FST without altering the locomotor performance in the OFT. Treatment with $(PhSe)_2$ ameliorated performance in the FST without altering the crossing numbers in the OFT. The inhibition of Na^+K^+ ATPase activity caused by malathion was prevented by treatment with $(PhSe)_2$. Exposure to malathion did not alter parameters of oxidative stress as well as AChE and MAO activities in cerebral cortex of rats. In conclusion, $(PhSe)_2$ exerted antidepressant-like effect in rats exposed to malathion. Na^+K^+ ATPase activity is, at least in part, involved in $(PhSe)_2$ antidepressant-like behavior.

The extensive use of pesticides for plant and crop protection in agriculture and the intensive development of new chemicals for this purpose have dramatically increased the variety and quantities of agrochemicals present in the environment. Organophosphorus pesticides (OPs) represent the most applied group of insecticides for the last two decades [9,27].

The malathion [S-1,2(bis-ethoxycarbonyl)ethyl O,O-dimethyl phosphorodithioate] is one of the most widely used OP for agriculture corps against several pests and public health programs [2,15]. Malathion is known to induce excitotoxicity through its bioactivated analog, malaoxon [24]. Moreover, studies have demonstrated that exposure to this OP causes oxidative stress [22,4].

In addition, an important sequel observed in acute poisoned patients with OP compounds is impairment in neurobehavioral performance, emotional *status* [10] and neurotoxicity caused by inhibition of acetylcholinesterase (AChE) activity [8]. Moreover, long-term exposure to low levels of OPs may produce neuropsychiatric symptoms including affective disorder such as anxiety, depression [37,10,38].

Depression is a common disorder and a major cause of disability, and causes death both by suicide and due to raised rates of physical disorders [32]. The mechanism of depression is quite complex [40]. Although psychobiological research on depression has traditionally concentrated on the neurotransmitters, noradrenaline and 5-HT, the role of acetylcholine in emotional behavior has been studied. Some evidence on this topic suggests that dysfunction of cholinergic transmission is involved in the pathophysiology of depression [19,21].

There is evidence of rapidly increasing interest in the area of effective chemical antidepressants which rates of response and remission and with lower adverse-effect [39]. In this way, diphenyl diselenide $(PhSe)_2$, an organoselenium compound, could be an attractive target for treatment of depression due to be a non-toxic drug when acutely administered to rats and mice [42,43] at doses that has pharmacological effect [41]. Recently, our group of research reported that this compound exerts antidepressant-like and anxiolytic-like effects [43].

In this study we examine if repeated administration of $(PhSe)_2$ would exert antidepressant-like effect in rats concomitantly exposed to the low dose of malathion. The role of Na^+K^+ ATPase, acetylcholinsterase (AChE) and monoamine oxidase (MAO) activities and oxidative stress was investigated in cerebral cortex of rats.

All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and use of laboratory animals (NIH publication 8023, revised 1996) and with the approval of the local Animal Use Committee. All chemicals were

* Corresponding author. Tel.: +55 55 3220 8140; fax: +55 55 3220 8978.
E-mail address: criswn@quimica.ufsm.br (C.W. Nogueira).

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obtained from Sigma (St. Louis, MO, USA) and Aldrich Chemical Co. (USA). $(PhSe)_2$ was prepared in our laboratory according to Paulmier [31] and the chemical purity (99.9%) was determined by GC/HPLC. Malathion 500 CE (50% purity, Indol do Brazil Agroquímica LTDA, Curitiba, PR, Brazil) was obtained from commercial grade. Male adult rats, weighing 200–300 g, were obtained from a local breeding colony. The animals were kept in separate animal rooms, on a 12-h light/dark cycle, in an air conditioned room ($22 \pm 2\,^{\circ}C$). Commercial diet (GUABI, RS, Brazil) and tap water were supplied *ad libitum*.

Rats were divided into four groups of 7–9 animals each. The group I received canola oil (1 ml/kg) + saline (1 ml/kg), group II received $(PhSe)_2$ (50 mg/kg) + saline (1 ml/kg), group III received canola oil (1 ml/kg) + malathion (50 mg/kg) and group IV received $(PhSe)_2$ (50 mg/kg) + malathion (50 mg/kg). Rats were treated once a day for 3 consecutive days with malathion and $(PhSe)_2$. Treatment with $(PhSe)_2$ was given immediately after malathion injection. $(PhSe)_2$ was dissolved in canola oil and administered per oral route (p.o.). Malathion was dissolved in saline 0.9% and administered intraperitonially (i.p.). The dosage regimen was based on Brocardo et al. [6] and did not produce overt signs of toxicity in rats [6]. At the 4th day, rats were submitted to the forced swimming test (FST) and open-field test (OFT). After that, rats were killed by decapitation and the cerebral cortex was immediately excised. The samples of tissues were homogenized in 50 mM Tris–HCl, pH 7.4 (1/3, w/v) and centrifuged at $2400 \times g$ for 15 min at $4\,^{\circ}C$. The low-speed supernatants (S_1) were separated and used for biochemical assays excepting for AChE and MAO activities.

The FST was conducted using the method of Porsolt et al. [33], with minor modifications. Behavioral naive rats were individually forced to swim in open plastic cylinders (height 40 cm, diameter 30 cm) containing 25 cm of water, maintained at $25 \pm 1\,^{\circ}C$. The pretest session was carried out 30 min after the last malathion and/or $(PhSe)_2$ treatment. In the pretest session, rats were allowed to swim for 15 min and then returned to their home cages. In the test session, 24 h later, rats were again submitted to the FST, with immobility time measured during 5 min. Each rat was judged to be immobile when it remained floating motionless in the water, making only those movements necessary to keep its head above water.

To assess the possible effects of malathion and $(PhSe)_2$ on the locomotor and exploratory activities, rats were evaluated in the OFT. The OFT was made of polywood and surrounded by walls 30 cm in height. The floor of the open-field, 45 cm in length and 45 cm in width, was divided by masking tape markers into 09 squares (3 rows of 3). Each animal was placed individually at the center of the apparatus and observed for 4 min to record the locomotor (number of segments crossed with the four paws) and exploratory activities (expressed by the number of time rearing on the hind limbs) [46].

The biochemical assays, thiobarbituric acid reactive species (TBARS) levels, non-enzymatic antioxidant defenses (non-protein thiols (NPSH) and ascorbic acid levels), enzymatic antioxidant defenses (catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST) activities), Na^+K^+ ATPase, acetylcholinsterase (AChE) and monoamine oxidase (MAO) activities were carried out.

TBARS levels, a measure of lipid peroxidation, were determined as described by Ohkawa et al. [29]. An aliquot of S_1 was incubated with 0.8% thiobarbituric acid (TBA), acetic acid buffer pH 3.4 and 8.1% sodium dodecil sulphate (SDS) at $95\,^{\circ}C$ for 2 h. The color reaction was measured at 532 nm. TBARS levels were expressed as nmol MDA/mg protein.

NPSH levels were determined by the method of Ellman [13]. An aliquot of S_1 was mixed (1:1) with 10% trichloroacetic acid (TCA) and centrifuged at $4000 \times g$ for 10 min. After the centrifugation, the protein pellet was discarded and free –SH groups were determined in the clear supernatant. An aliquot of supernatant was added in 1 M potassium phosphate buffer, pH 7.4, and 10 mM 5,5-dithiobis(2-nitrobenzoic acid) (DTNB). The color reaction was measured at 412 nm. NPSH levels were expressed as μmol NPSH/g tissue.

Ascorbic acid determination was performed as described by Jacques-Silva et al. [25]. Proteins were precipitated in 10 volumes of a cold 5% TCA solution. An aliquot of the sample at a final volume of 1 ml of the solution was incubated at $37\,^{\circ}C$ for 3 h then H_2SO_4 65% (v/v) was added to the medium. The reaction product was determined using a color reagent containing 4.5 mg/ml dinitrophenyl hydrazine and $CuSO_4$ (0.075 mg/ml) at 520 nm. The content of ascorbic acid was expressed as μmol ascorbic acid/g tissue.

CAT activity was assayed spectrophotometrically by the method of Aebi [1], which involves monitoring the disappearance of H_2O_2 in the homogenate presence at 240 nm. Enzymatic reaction was initiated by adding an aliquot of S_1 and the substrate (H_2O_2) to a concentration of 0.3 mM in a medium containing 50 mM potassium phosphate buffer, pH 7.0. The enzymatic activity was expressed in units (U)/mg protein (1 U decomposes 1 μmol of H_2O_2 per minute at pH 7 at $25\,^{\circ}C$).

GPx activity was assayed spectrophotometrically by the method of Wendel [47], through the GSH/NADPH/glutathione reductase system, by the dismutation of H_2O_2 at 340 nm. S_1 was added in GSH/NADPH/glutathione reductase system and the enzymatic reaction was initiated by adding H_2O_2. In this assay, the enzyme activity is indirectly measured by means of NADPH decay. H_2O_2 is decomposed, generating GSSG from GSH. GSSG is regenerated back to GSH by glutathione reductase presents in the assay media at the expenses of NADPH. The enzymatic activity was expressed as nmol NADPH/min/mg protein.

GR activity was determined as described by Calberg and Mannervik [7]. In this assay, GSSG is reduced by GR at the expense of NADPH consumption, which is followed at 340 nm. GR activity is proportional to NADPH decay. The enzymatic activity was expressed as nmol NADPH/min/mg protein.

GST activity was assayed spectrophotometrically at 340 nm by the method of Habig et al. [23]. The reaction mixture contained an aliquot of S_1, 0.1 M potassium phosphate buffer pH 7.4, 100 mM GSH and 100 mM CDNB, which was used as substrate. The enzymatic activity was expressed as nmol CDNB conjugated/min/mg protein.

For Na^+K^+ ATPase activity assay, it was used a reaction mixture containing S_1, 3 mM MgCl, 125 mM NaCl, 20 mM KCl and 50 mM Tris–HCl, pH 7.4, in a final volume of 500 μl. The reaction was initiated by the addition of ATP to a final concentration of 3.0 mM. Control samples were carried out under the same conditions with the addition of 0.1 mM ouabain. The samples were incubated at $37\,^{\circ}C$ for 30 min, the incubation was stopped by adding TCA solution (10%) with 10 mM $HgCl_2$. Na^+K^+ ATPase activity was calculated by the difference between the two assays. Released inorganic phosphate (Pi) was measured by the method of Fiske and Subbarow [16]. Enzyme activity was expressed as nmol Pi/mg protein/min.

For AChE activity assay, the samples of cerebral cortex were homogenized in 0.25 M sucrose buffer (1/10, w/v) and centrifuged at $2400 \times g$ at $4\,^{\circ}C$ for 15 min. Activity of AChE was carried out according to the method of Ellman et al. [14], using acetylthiocholine as substrate. The activity of AChE was spectrofotometrically measured at 412 nm. The activity of AChE was expressed as nmol/min/mg protein.

A preparation of cortex mitochondria was used for MAO assay as described by Soto-Otero et al. [45]. Cerebral cortices were immediately removed and washed in ice-cold isolation medium (pH 7.4, Na_2PO_4/KH_2PO_4 isotonized with sucrose). Mitochondria from cortex were then obtained by differential centrifugation. Briefly, after removing blood vessels and pial membranes, cerebral cortices were manually homogenized with four volumes (w/v) of the isolation medium. Then, the homogenate was centrifuged at $900 \times g$