EFFECTS OF 3,5-DIBROMO-L-PHENYLALANINE IN ANIMAL MODELS OF STROKE, SEIZURES AND SENSORIMOTOR GATING DEFICIT

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Anatolii Martyniuk *received Ph.D. (1983) and D.Sc. (1993) degrees at the Bohomolets Institute of Physiology in Kyiv, Ukraine under the mentorship of Dr. Platon Kostyuk. In 1993-1996 Dr. Martynyuk completed his postdoctoral training in the laboratory of Drs. Andrew Rankin and Kathy Kane at the University of Glasgow in Glasgow, United Kingdom. In 1996 he moved to the United States. He is a graduate faculty and a principal investigator at the Department of Anesthesiology and the McKnight Brain Institute, University of Florida, Gainesville, Florida. Dr. Martynyuk's current research predominantly focuses on the contribution of abnormal glutamatergic activity to the etiology of CNS disorders, such as stroke, epileptic seizures, and schizophrenia, and on drug development for their treatments. The most recently initiated project in his laboratory aims at validating the hypothesis that GABAA receptor enhancing general anesthetics cause epileptogenic and neurotoxic effects and delayed impairment of cognition in neonates and infants.*

3,5-DBr-L-Phe traps hydroxyl radicals (Fig. 1), acts as a partial NMDA receptor agonist (Fig. 2), depresses activity of AMPA/kainate receptors (Fig. 3, *a*) and glutamate release (Fig. 3, *b*).

3,5-DBr-L-Phe decreases the neurological deficits and infarct volume caused by MCAo (Fig. 4).

3,5-DBr-L-Phe reduces seizures induced by PTZ (Fig. 6).

3,5-DBr-L-Phe does not alter arterial blood pressure and heart rate (Fig. 7).

3,5-DBr-L-Phe does not affect activation of neuronal caspase-3 in the rat cortex (Fig. 8).

3,5-DBr-L-Phe prevents disruption of PPI of startle caused by stroke or MK-801 (Fig. 9).

In summary, 3,5-DBr-L-Phe produced efficacious neuroprotection in a rat model of stroke caused by intracerebral injection of ET-1 adjacent to the MCA, reduced seizures induced by PTZ, and decreased the PPI deficit caused by both

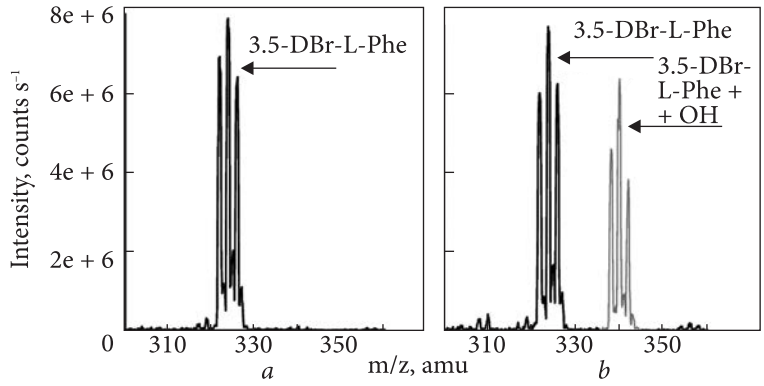

Fig. 1. Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analysis of 3,5-DBr-L-Phe before (*a*) and 1 hour after initiating the Fenton reaction (*b*). Mass spectral fragmentations of the [M-H] + ions of 3,5-DBr-L-Phe and of 3,5-DBr-L-Phe and its Fenton reaction product, 3,5-DBr-L-Phe + ·OH, (*b*) were taken with atmospheric pressure chemical ionization (APCI), in positive ion mode on a triple quadrupole mass spectrometer. Phe + ·OH (*b*) were taken with atmospheric pressure chemical ionization (APCI), in positive ion mode on a triple quadrupole mass spectrometer

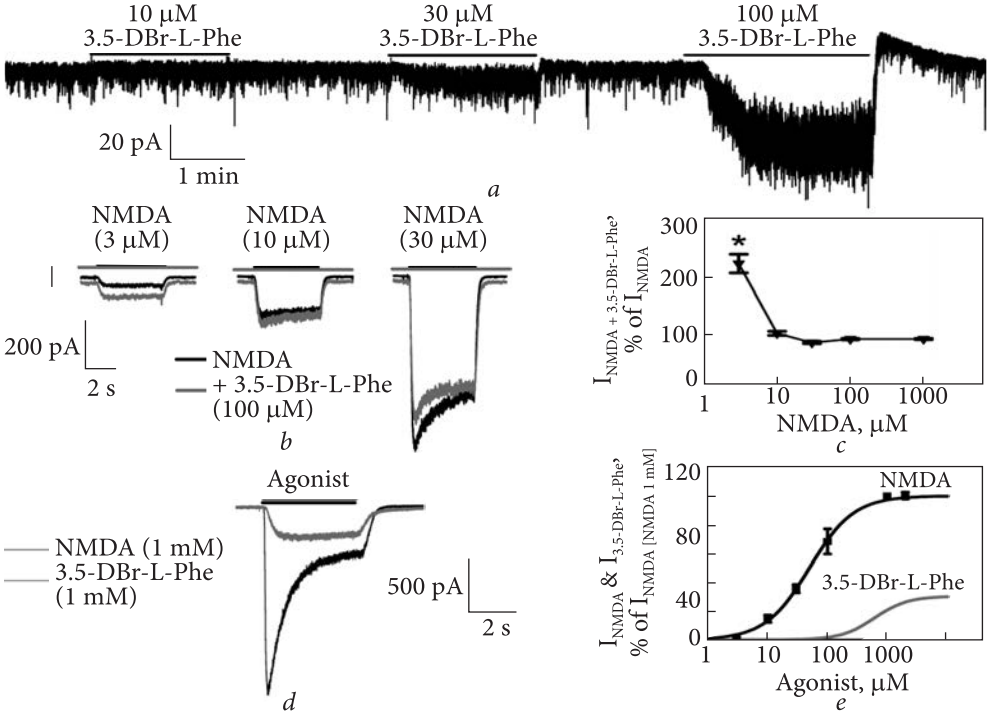

Fig. 2. Properties of 3,5-DBr-L-Phe-activated current: *a* — Example of NMDA mEPSCs recorded in the presence of different concentrations of 3,5-DBr-L-Phe; *b* and *c* — Activating effect of 3,5-DBr-L-Phe on NMDA current depends on concentration of NMDA; *d* — Examples of NMDA (1 mM) and 3,5-DBr-L-Phe (1 mM) activated currents recorded from the same neuron; *e* — Concentration-response curves to NMDA and 3,5-DBr-L-Phe were obtained for rat cerebrocortical cultured neurons in the presence of 10 μM glycine

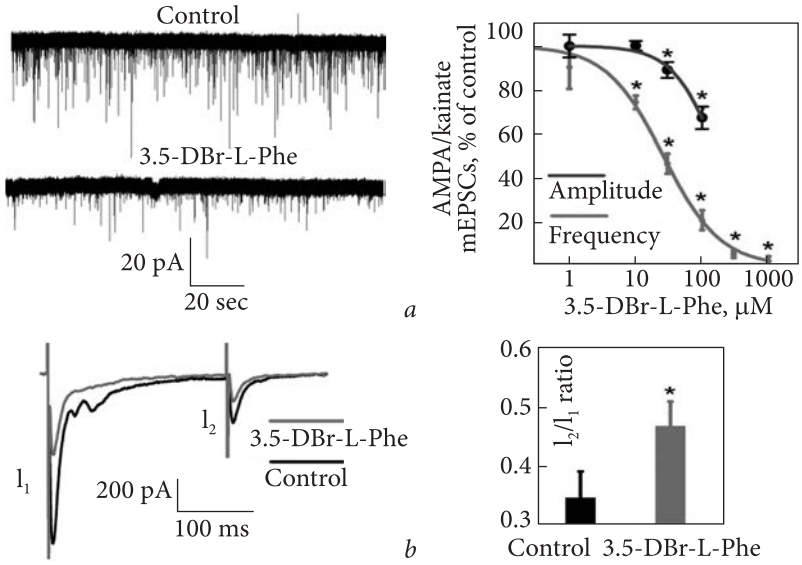

Fig. 3. 3,5-DBr-L-Phe depresses AMPA-kainate mEPSCs and glutamate release in rat cerebrocortical cultured neurons: *a* — Representative traces of AMPA-kainate mEPSCs and concentration-response relationships for 3,5-DBr-L-Phe to attenuate AMPA/kainate mEPSC frequency and amplitude; *b* — Effect of 3,5-DBr-L-Phe on the evoked EPSCs. Synaptic responses were evoked by applying two subthreshold electric stimuli (0.4-1 ms, 50-90 V, 250 ms apart). The amplitudes of the 1st and 2nd EPSCs were measured against the baseline; each point represents an average of five subsequent sweeps

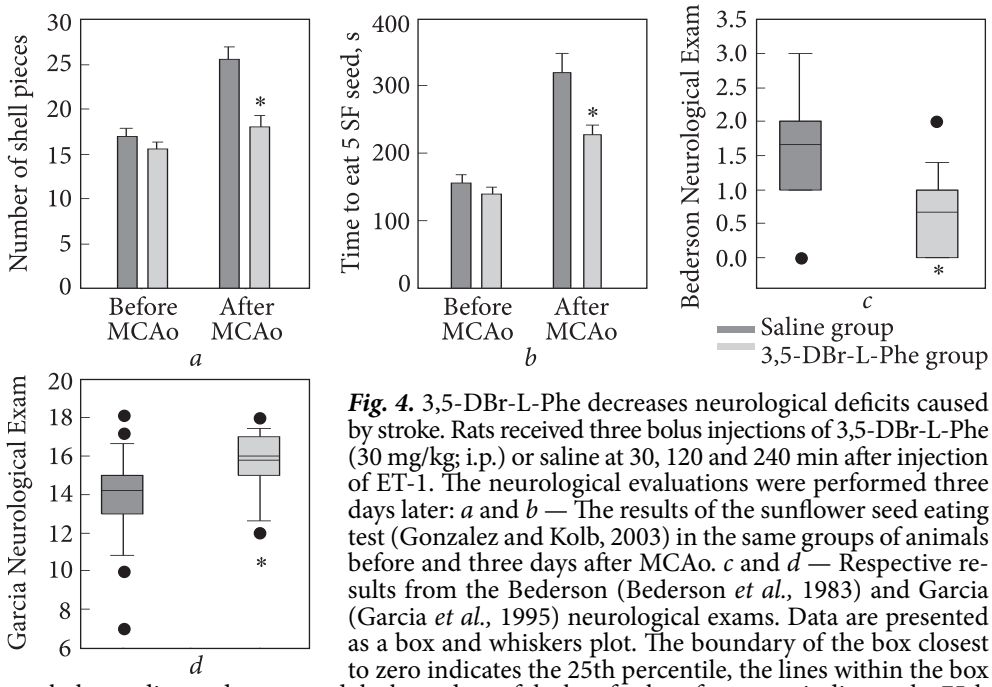

Fig. 4. 3,5-DBr-L-Phe decreases neurological deficits caused by stroke. Rats received three bolus injections of 3,5-DBr-L-Phe (30 mg/kg; i.p.) or saline at 30, 120 and 240 min after injection of ET-1. The neurological evaluations were performed three days later: *a* and *b* — The results of the sunflower seed eating test (Gonzalez and Kolb, 2003) in the same groups of animals before and three days after MCAo. *c* and *d* — Respective results from the Bederson (Bederson *et al.,* 1983) and Garcia (Garcia *et al.,* 1995) neurological exams. Data are presented as a box and whiskers plot. The boundary of the box closest to zero indicates the 25th percentile, the lines within the box mark the median and mean, and the boundary of the box farthest from zero indicates the 75th percentile. The whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. Outliers are represented as individual data points. *, $P < 0.05$ relative to the saline-treated animals, n = 15 (3,5-DBr-L-Phe) and n = 8 (saline)

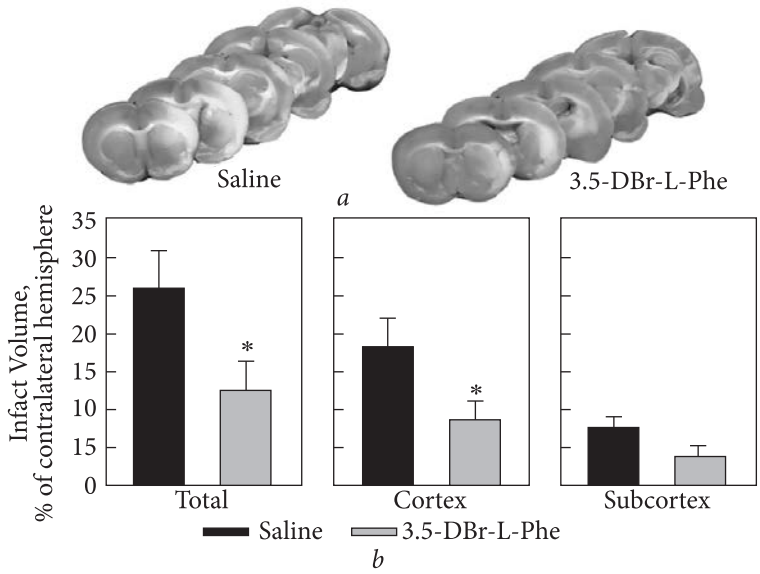

Fig. 5. 3,5-DBr-L-Phe decreases the volume of infarcted brain tissue caused by intracerebral injection of ET-1. Results obtained from the same rats as in Fig. 4. Histopathological analysis of the infarcted brain tissue was performed 3 days after administration of ET-1 using 2% 2,3,5-triphenyltetrazolium chloride (TTC) staining. *a* — TTC-stained sections of brain at 5 coronal levels from representative rats which received either saline or 3,5-DBr-L-Phe. *b* — Total volume of infarcted brain tissue as well as volume of infarcted brain tissue in the cortex and in the subcortex presented as a percent of the contralateral hemisphere. *, $P < 0.05$ vs 0.9% Saline

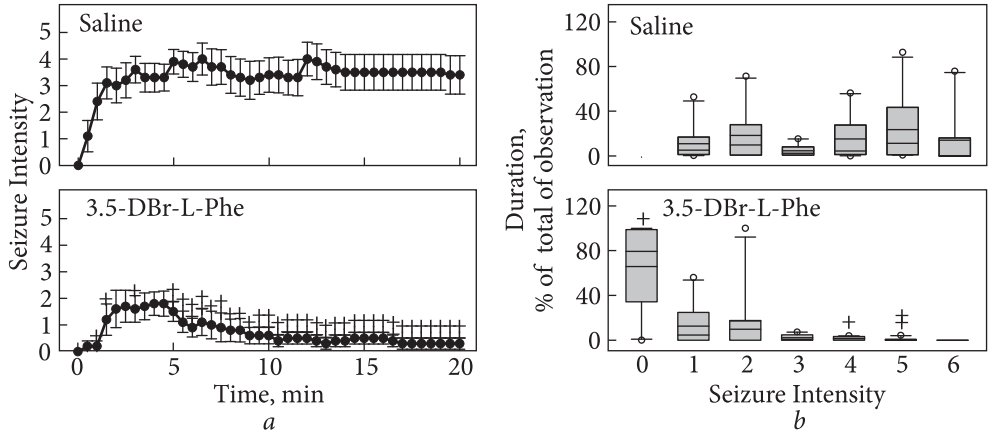

Fig. 6. 3,5-DBr-L-Phe depresses PTZ-induced seizures. Each group of animals received an injection of 3,5-DBr-L-Phe (30 mg/kg, i.p.) or equal volumes of saline 15 min prior to PTZ administration (60 mg/kg, i.p.): *a* — Seizure intensity at a given time (bin interval 30 s) during the 20 min observation period. The severity of seizures was scored using a 7-point behavioral seizure score: 0, no response; 1, ear and facial twitching; 2, convulsive waves through the body; 3, myoclonic jerks and/or rearing; 4, clonic-tonic seizures; 5, generalized clonic-tonic seizures, loss of postural control; 6, lethal. Data are presented as mean ±SE. *b* — Box plots showing the duration of seizures of a given intensity (score) during the experiment. $^{+}$, $P < 0.05$ and ‡, $P < 0.01$ vs saline for both (*a*) and (*b*), n = 10 per group

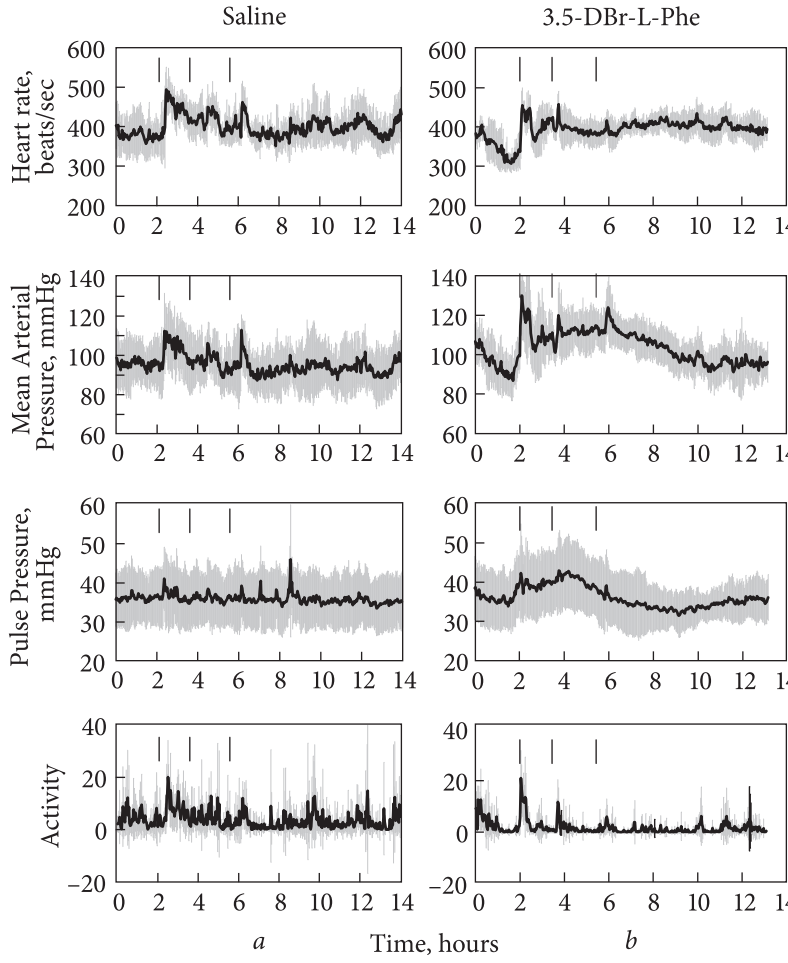

Fig. 7. Effects of 3,5-DBr-L-Phe on cardio-vascular parameters and locomotor activity. Cardiovascular parameters and loco-motor activity in conscious rats were measured using Rat telemetry transducers (DSI, St. Paul, MN, USA) implanted into the abdominal aorta. Arrows indicate time points at which 3,5-DBr-L-Phe (30 mg/kg, i.p., right panel) or equal volume of saline (left panel) was administered. Data are averaged (black circles) ±SE (gray bars) from 6 rats for each treatment group

stroke and MK-801. Importantly, significant neuroprotection with 3,5-DBr-L-Phe could be achieved by administering this agent after the onset of stroke. Thus, the type of action that 3,5-DBr-L-Phe produces can be useful for the treatment of certain neurological and neuropsychiatric disorders.

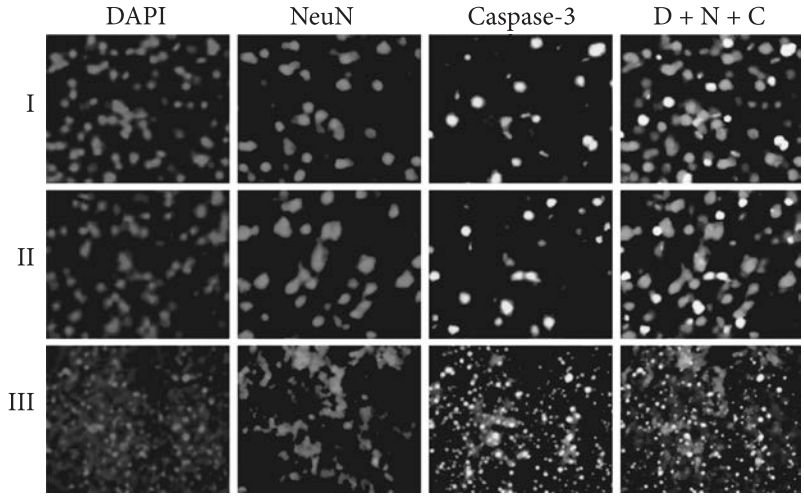

Fig. 8. Representative 40x images of the nonischemic cortexes from the contralateral hemisphere of rats exposed to MCAo. The rats received three boluses i.p. of 3,5-DBr-L-Phe (I) 30 mg/kg each or equal volume of saline (II) as described in Fig. 4. Immunohistochemical analysis was performed three days after the onset of MCAO; all cells (DAPI); neurons (NeuN); activated caspase 3 signal; D + N + C — an overlay of images. (III), representative images from the cortex of ipsilateral hemisphere (stroke injured area) of a saline-treated rat

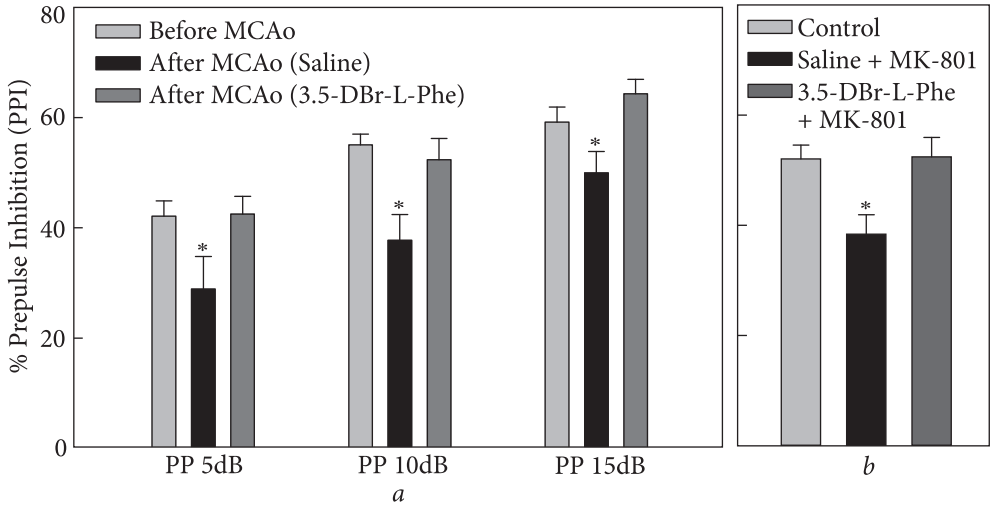

Fig. 9. The PPI test was performed one day prior and three days after the onset of MCAo (*a*). The animals were treated with 3,5-DBr-L-Phe (n = 15) or saline (n = 8) as described in *b* — PPI was measured in all rats before treatment (Control, n = 34) and after administration of: 1) saline and MK-801 (0.15 mg/kg) (n = 17) or 2) 3,5-DBr-L-Phe (30 mg/kg) and MK-801 (0.15 mg/kg) (n = 17). *, $P < 0.05$ relative to the control group. The average %PPI data for all three prepulse intensities for each treatment group is shown in *b*. An SR-LAB apparatus and accompanying software (San Diego Instruments, San Diego, CA, USA) were used to perform the tests. %PPI = = 100 × [(pulse alone) — (prepulse + pulse)]/pulse alone (Geyer and Dulawa, 2003)

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