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Physiology & Behavior 81 (2004) 91–99

PHYSIOLOGY & BEHAVIOR

Participation of the substantia nigra dopaminergic neurons in the occurrence of gastric mucosal erosions

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Received 9 April 2003; received in revised form 27 August 2003; accepted 13 January 2004

Abstract

Previous reports indicate that dopaminergic systems play an important role on gastric mucosal erosions. In the present study, the participation of intrinsic neurons of the substantia nigra (SN) and ventral tegmental area (VTA) on the occurrence of stomach ulceration was investigated. It was found that bilateral microinfusions of a neurotoxic dose (20 μg/μl) of *N*-methyl-D-aspartate (NMDA) into the SN, but not in the VTA, lead to gastric erosions 24 h after the surgery. A decrease in dopamine levels in the caudate 24 h after the microinfusion of NMDA into the SN was also observed. Destruction of SN cell bodies with 6-hydroxydopamine (6-OHDA) did not induce gastric ulceration or changes in dopamine levels in the caudate nucleus 24 h after the lesioning procedure. NMDA neurotoxicity is mediated by the acute excitatory or activational effects, in contrast to 6-OHDA, suggesting that the occurrence of gastric ulceration after the infusion of NMDA into the SN is not due to the cell death per se but is related to an overactivation of these cells that precede their death. Taken together, these results suggest that modulation of dopaminergic levels by neurons located within the SN may play an important role for the development of gastric erosions.

Keywords: Gastric ulceration; Substantia nigra; Ventral tegmental area; NMDA; 6-OHDA

1. Introduction

Considerable evidence indicates that dopaminergic systems play an important role in several gastrointestinal functions [14–17,19,29,32,35]. In addition, both central and peripheral dopamine activities are affected by stress and appear to modulate the consequences of stress exposure on gastroduodenal injury. In general, experimental stressors increase dopamine turnover in certain brain regions (e.g., frontal cortex), whereas the central administration of dopamine agonists reduce and dopamine antagonists augment gastric ulcer formation induced by stressors [15].

There are three major dopaminergic pathways in the central nervous system. The nigrostriatal pathway consists mainly of cell bodies in the pars compacta of the substantia nigra (SN) and sends axons primarily to the caudate putamen and the central nucleus of the amygdala [3,6,39]. The mesolimbic pathway originates in cell bodies in the ventral tegmental area (VTA) that project axons ascending together with the nigrostriatal dopaminergic axons and terminate in different areas in the forebrain, including the nucleus accumbens, olfactory turbeculum, septal complex, and frontal cortex [36]. Finally, the mesocortical pathway also has its origins in the VTA but contains ascending projections that follow a more medial route [39].

It has been reported that both VTA and SN play a role on gastric erosion formation. For example, bilateral lesions of the VTA and SN increase stress-induced ulceration, whereas VTA lesions also lead to gastric erosions in control nonstressed rats [32]. Moreover, electrolytic lesions of the SN produce gastric erosions 24 h after surgery [34]. However, the electrolytic lesioning procedure causes damage to both cell bodies and axons of passage. This is an important issue because lateral hypothalamic cell bodies, involved in the occurrence of gastric erosions, send descending axons that travel dorsal to the SN and across the VTA [5,23,26,42]. Therefore, the

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doi:10.1016/j.physbeh.2004.01.004

participation of SN or VTA on gastric erosions is apparently not related to destruction of intrinsic neurons in either of these two brain structures but is due to a disruption of projections of descending fibers, possibly originating in the lateral hypothalamus.

The excitatory amino acid *N*-methyl-D-aspartate (NMDA) has been described as a highly specific neurotoxic agent. Prolonged stimulation of the NMDA receptor eventually results in cell death sparing axonal fibers of passage [21]. It has been reported that SN and VTA have a high concentration of NMDA receptors and modulate the firing activity of dopaminergic neurons [9,27]. In fact, NMDA antagonist microinjected into the SN or VTA attenuates the increased release of DA in the striatal area that occurs when animals are exposed to stressful stimuli [7]. Therefore, the first experiment investigated the influence of intrinsic neurons in the SN and VTA on the incidence of gastric erosions 24 h after the bilateral microinfusions of a neurotoxic dose of NMDA into these two brain areas.

2. Experiment 1

2.1. Methods

2.1.1. Subjects

Twenty male albino Sprague–Dawley (Bantin & Kingman, Fremont, CA) rats weighing 230–280 g served as subjects. Animals were housed individually in a colony room with a 12:12-h light/dark cycle and had ad lib access to food and water at least 1 week prior to the beginning of the experiment.

2.1.2. Surgery and procedure

Rats were food deprived overnight (approximately 8–10 h) and then anesthetized with sodium pentobarbital (Nembutal; 65 mg/ml ip) and mounted in a Kopf model 900 stereotaxic instrument with the skull oriented in the horizontal plane. One group of animals (SN, $n=9$) received two bilateral infusions of 1 μl of NMDA (Sigma, St. Louis, MO) dissolved in 0.1 M phosphate-buffered saline (PBS; pH 7.4) in adjacent sites within the SN. The NMDA dose was 20 μg/μl and the drug was made fresh. With reference to the Paxinos and Watson atlas [31], injections were placed 5.3 mm posterior to bregma, 2.6 and 2.0 mm lateral to each side of the midline, and 6.9 and 7.3 mm, respectively, ventral to dura. A second group (VTA, $n=6$) received single bilateral injections of 1 μl of NMDA (20 μg/μl) in the VTA region with stereotaxic coordinates set to 4.8 mm posterior to bregma, 0.8 mm lateral to each side of the midline, and 8.1 mm ventral to dura. Although the dose of NMDA infused bilaterally into the SN and VTA was the same (20 μg/μl), the SN group was given a larger total volume of NMDA than the VTA group (SN, 2 μl; VTA, 1 μl). This was done because the SN constitutes a larger brain area than the VTA, and to affect as many SN cells as possible with NMDA, adjacent 1 μl infusions were given, as noted above. Animals in the control (CONT) group received PBS vehicle injections in either the SN ($n=3$) or the VTA ($n=2$).

Infusion was made by a glass micropipette connected to a 10 μg/μl Hamilton microsyringe via a PE tubing. The Hamilton syringe was mounted on an injection pump set up to deliver 1 μl of the drug over 15 min. Following the infusion, the micropipette was kept in place for an additional 5 min to prevent the drug from diffusing up the micropipette track. Before and after each injection, the flow at the tip of the micropipette was verified by turning the pump on until a droplet appeared. Control animals were infused with an equal volume of PBS vehicle alone.

After surgery, animals were returned to their home cages and were food deprived for an additional 24 h. This additional postoperative food deprivation period was employed so that the rate of gastric erosion formation would not be influenced by presence of food in the stomach in some animals but not in others. Following the postoperative deprivation period, animals were given a lethal injection of sodium pentobarbital (100 mg/kg ip) and sacrificed by decapitation. A ligature was placed around the duodenum and the esophagus, and approximately 3 ml of 10% formalin were infused into the stomach through the esophagus. Ten minutes later, the stomach was opened along the great curvature rinsed gently with water and spread on a flat surface and fixed with 10% formalin. The stomachs were stored in formalin for several weeks, and after this time, the gastric mucosa was examined with a binocular dissection microscope at $\times 8$. One eyepiece was fitted with a reticle, permitting gastric lesions to be quantified in terms of total area (mm^2). Any discontinuity in the gastric mucosa was considered a gastric erosion. An independent rater who was blind to the experimental conditions examined all stomachs.

Brains of all animals were removed and stored in 10% formalin for about 1 week, sectioned using the cryostatic method at 50–60 μm, mounted on slides, and stained with thionine. The extent of brain damage (neuronal cell loss) produced by the microinjection of the NMDA was evaluated with the reference to the Paxinos and Watson [31] rat brain atlas. Data obtained from animals with misplaced NMDA injections were excluded from statistical analysis.

2.1.3. Statistical analysis

Results for gastric ulceration are presented as means ± S.E.M. Because group means and variances tended to covary, and the distributions were positively skewed, a $\log_{(X+1)}$ transformation was conducted on the gastric erosion raw data for each animal. A one-way analysis of variance (ANOVA) was used to detect overall differences followed by Fisher's Least Significant Difference Post Hoc Tests to determine specific differences between groups.

2.2. Results and discussion

2.2.1. Histology

Three animals were excluded from the experiment due to death during surgery or infusion misplacement. The final size of each group was as follows: SN = 7, VTA = 5, and CONT = 5 (which had PBS vehicle infused into the SN or VTA). A representative brain section from an animal in the SN group is shown in Fig. 1. Lesions tended to be symmetrical and were in both pars compacta and pars reticularis of the SN. Damage frequently extended to the parabrachial nucleus, peripeduncular nucleus, minimus nucleus, retroenthmoid nucleus, posterior intralaminar thalamic nucleus, and posterior areas of the VTA. Some animals ($n = 3$) had more extensive lesion encompassing the red nucleus, deep mesencephalic nucleus, anterior pretectal nucleus, and some portions of the medial geniculate body.

A representative brain section from an animal in the VTA group is shown in Fig. 2. Lesions tended to be symmetrical and damaged most of the VTA region with some destruction of the caudal portion of the LH, posterior hypothalamic area, visual tegmental nucleus, relay zone, parabrachial pigmented nucleus, and paranigral nucleus.

2.2.2. Volume of tissue damage and cell count

Mean volume of brain tissue affected was as follows: SN = 7.84 ± 0.51 mm^3, VTA = 5.50 ± 0.56 mm^3, and CONT = 0.18 ± 0.04 mm^3. ANOVA indicated an overall difference among the groups [$F(2,14) = 66.2$, $P < .0001$]. Post hoc comparisons revealed that both SN and VTA groups infused with NMDA had a greater volume of brain tissue affected than the CONT group (all Ps $< .01$). Additionally, the SN group had significantly more tissue affected than the VTA group ($P < .01$). This difference in volume between the SN and the VTA groups was expected because, as noted previously, the SN group was given a larger amount of NMDA to compensate for its larger size as compared with the VTA group. In either case, a major portion of each brain structure displayed apparent cell loss. Representative areas of smallest and largest regions affected by NMDA are shown in Fig. 3 for the SN and in Fig. 4 for the VTA.

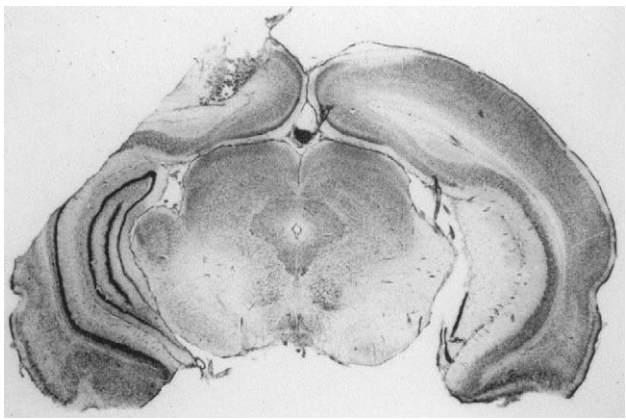

Fig. 1. Histological section taken through the midpoint of a representative NMDA (20 μg/μl) infusion into the SN (thionine stain).

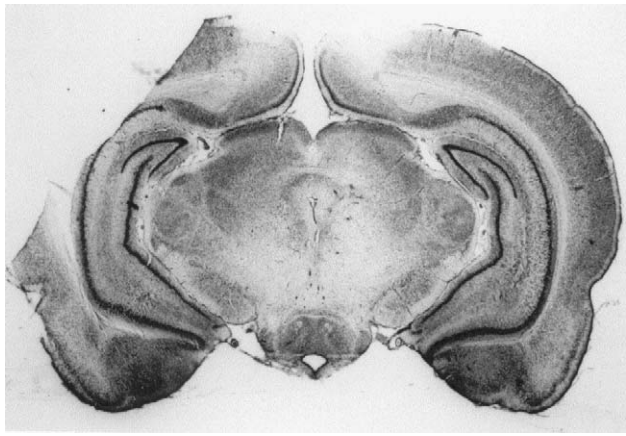

Fig. 2. Histological section taken through the midpoint of a representative NMDA (20 μg/μl) infusion into the VTA (thionine stain).

To get ordinal index of the extent of cellular involvement after the NMDA infusion into the SN and VTA, cells were counted in each side of the brain in the region of maximal expression of apparent cell lost (i.e., midpoint of the infusion site) and equivalent area in the CONT group. This procedure, described in detail elsewhere [24], consisted in counting the all-stained cells defined as a blue dot within an area of 1.0×1.0 mm at a magnification of $\times 100$. Fig. 5 presents the results for cell count. All animals in the CONT groups (vehicle injection into either SN or VTA) had no apparent cell loss and were not different from each other. NMDA lesions in either SN or VTA lead to a reduction in the number of cells. NMDA lesions in the SN destroyed approximately 50% of cells (50.7% in the right hemisphere and 50.1% in the left hemisphere), whereas NMDA lesions in the VTA destroyed about 37% of cells (35.6% in the right hemisphere and 38.9% in the left hemisphere). ANOVA revealed an overall difference among the groups [$F(3,18) = 40.7$, $P < .001$ for the right hemisphere and $F(3,18) = 62.7$, $P < .001$ for the left hemisphere]. Post hoc comparisons demonstrated a reliable difference between the NMDA lesion in both structures in comparison with the CONT groups in both hemispheres (all Ps $< .005$). A reliable difference between the VTA and the SN NMDA lesion groups indicate that SN lesions were reliably larger than those produced in the VTA. The difference in the cell count number between the SN and the VTA groups is probably due to the difference in the amount of NMDA injected in each of the structures.

2.2.3. Gastric erosions

Animals injected with PBS vehicle in either the SN or the VTA exhibited relatively normal-looking stomachs. Therefore, results from the two control groups were pooled for the purpose of statistical analysis. Fig. 6 shows the mean total area of stomach erosions observed in the SN, VTA, and CONT groups. ANOVA revealed a reliable main effect

among groups [$F(2,14) = 6.28$, $P = .011$]. Post hoc comparisons indicated that rats in the SN group had significantly more gastric erosions that those in either the VTA or the CONT group (all Ps $< .05$). Moreover, no significant difference in gastric erosions was found between VTA and CONT groups ($P > .4$). These results indicate that damage to neurons in the SN, but not in the VTA, produces gastric mucosal damage 24 h after surgery. These results also replicate the findings of Roland and Grijalva [34], who showed that electrolytic lesions of the SN, but not of the VTA, reliably produced gastric erosions. The gastric erosions observed in the SN group were also similar to those noted by Roland and Grijalva. They were confined to the glandular portion of the stomach and were in gross appearance, typically spherical or oblong, and superficially covered with blood.

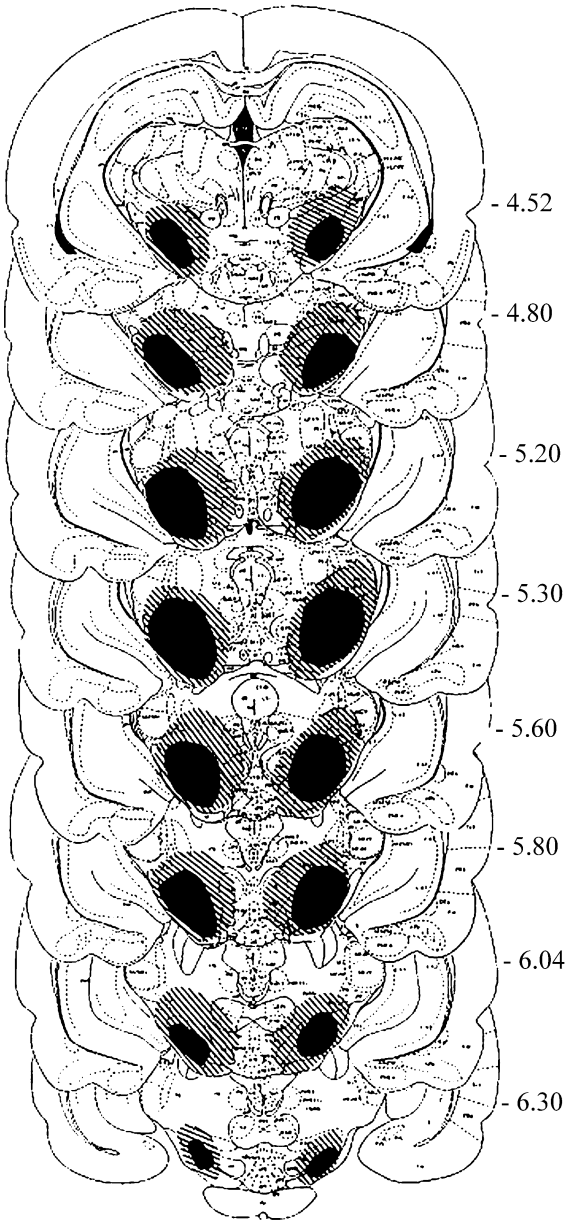

Fig. 3. Diagrammatic representation of the smallest (solid area) and largest (striped area) diffusion area produced by NMDA infusion in the SN. With reference to the Paxinos and Watson [31] atlas, the numbers on the right side of each plate indicate the distance (mm) from bregma.

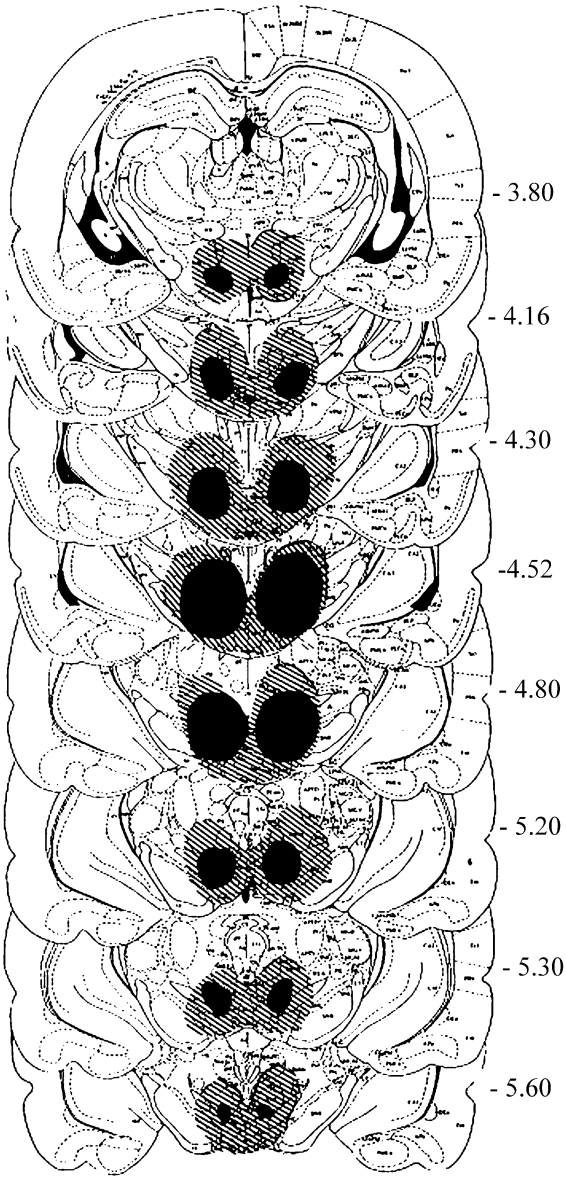

Fig. 4. Diagrammatic representation of the smallest (solid area) and largest (striped area) diffusion area produced by NMDA infusion in the VTA. With reference to the Paxinos and Watson [31] atlas, the numbers on the right side of each plate indicate the distance (mm) from bregma.

3. Experiment 2

Experiment 1 showed that a neurotoxic dose of NMDA infused into the SN induced stomach erosions within 24 h after surgery. Although the SN contains dopaminergic neurons highly sensitive to NMDA agonist [9], it not possible to conclude that gastric ulceration induced by

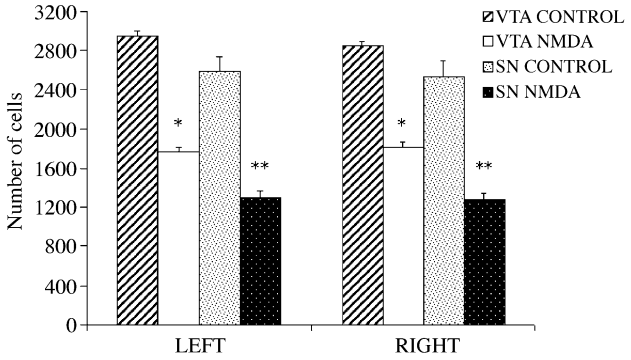

Fig. 5. Mean ± S.E.M. cell count in the left and right VTA and SN under high-power magnification 24 h after NMDA (20 μg/μl) or CONT (PBS) infusions. * $P<.05$ in comparison with VTA control; ** $P<.05$ in comparison with SN control.

NMDA infusion in the SN is mediated by dopaminergic mechanisms. The pars reticularis of the SN contains many GABAergic neurons with projections to the ventromedial nucleus of the thalamus [1,11], superior colliculus [41], and tegmental reticular formation [8]. This nigrotegmental projection innervates the pedunculopontine tegmental nucleus, dorsolateral portion of the central gray, deep mesencephalic nucleus, parabrachial nuclei, locus coeruleus, and dorsal and medial raphe nuclei [12]. Because NMDA lesions can also destroy GABAergic neurons [2,30,43], gastric ulceration induced by NMDA infusion in the SN is apparently not mediated by dopaminergic neurons but by some other neurochemical system. The second experiment addressed this issue by examining the effects of microinjections of a neurotoxic dose NMDA or the highly specific dopaminergic neurotoxin, 6-hydroxydopamine (6-OHDA), into the SN on gastric erosion formation. Additionally, dopamine levels in the caudate nucleus were measured through the high-pressure liquid chromatography (HPLC) technique 24 h after the 6-OHDA or NMDA microinfusions in the SN.

3.1. Methods

3.1.1. Subjects and procedure

Nineteen male rats weighing 220–270 g were employed as subjects. Animals were maintained as described previously in Experiment 1. Prior to surgery, all animals were food deprived but not water deprived overnight and then anesthetized. Bilateral NMDA lesions in the SN ($n=7$) were accomplished following the same procedure as in Experiment 1. Brain coordinates and general infusion procedures for bilateral 6-OHDA lesion ($n=6$) were the same as for the NMDA lesion group. The 6-OHDA lesions were induced by bilaterally infusing 8 μg/μl 6-OHDA hydrobromine (Sigma) dissolved in 0.2% of ascorbic acid. To protect noradrenergic neurons, rats receiving 6-OHDA were intraperitoneally injected with 15 mg/kg paragyline hydrochloride and 15 mg/kg desipramine hydrochloride (both from Sigma) 30 min prior to surgery. The CONT group ($n=6$) were subjected to the same procedure as the experimental groups, with the exception that PBS ($n=3$) or 0.2% of ascorbic acid ($n=3$) was infused into the SN.

3.1.2. Tissue dissection

Twenty-four hours after surgery, all animals were sacrificed by decapitation and brains were rapidly removed, frozen in powdered dry ice, and stored for 1–2 days at −80 °C prior to dissection. Brains were placed in a cold steel blocker and two 1 mm sections were cut for samples of caudate nucleus and frontal cortex. From these sections, samples of the caudate nucleus and frontal cortex were dissected and placed in a 300 μl of 0.1 M perchloric acid solution containing 10 μM ascorbate, 10 μM EDTA, and DHBA (10^{-5} M) for an internal standard. This sample was sonicated for 5 s and then centrifuged at 21,000 × g (4 °C) for 7 min. Supernatants were removed, transferred to Rainin filterfuge tubes (0.2 μm nylon 66 filters), and centrifuged for 2 min at 515 × g (4 °C). Protein pellets from first centrifugation were stored at −80 °C until analyzed for protein content using the Bradford assay.

3.1.3. HPLC analysis

Filtered samples were analyzed for dopamine content using HPLC electrochemical detection under isocratic conditions. During both assays, the samples were kept at 3 °C in a Gilson autosampler tray and 40 μl samples were injected onto a reversed-phase column (Rainin C-18, 25 cm, 5 μm) with a flow rate of 0.7 ml/min. The samples were processed using an ESA Coulochem II electrical chemical detection system (guard electrode = +0.7 V, oxidation electrode = +350 mV, and reduction electrode = −250 mV) with a mobile phase containing 0.1 M citric acid, 0.75 mM NA_2HPO_4, 1.0 mM EDTA, and 13% methanol (pH 4.0).

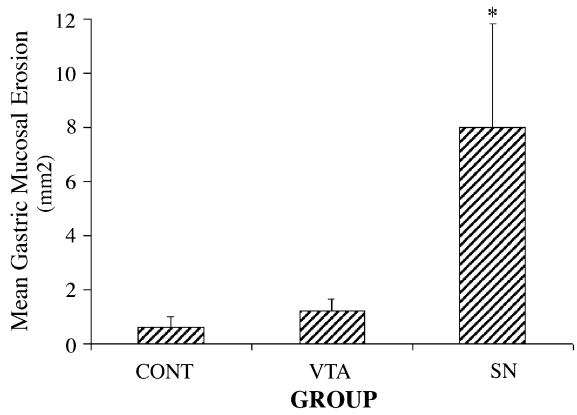

Fig. 6. Mean ± S.E.M. total area of gastric mucosal erosions in rats 24 h after bilateral NMDA (20 μg/μl) infusion in the SN or VTA. Control vehicle infusions (CONT) consisted of PBS microinjections into the SN ($n=3$) and VTA ($n=2$). * $P<.05$ compared with CONT group.

An EZchrom analysis program was used for acquisition and analysis of all sample data.

3.2. Results and discussion

3.2.1. Histology and cell count

Two animals were excluded from the experiment due to error during the HPLC analysis. The final size of each group was as follows: CONT = 6 (PBS = 3, ascorbic acid = 3), NMDA = 5, and 6-OHDA = 6. Control animals injected with either PBS or 0.2% of ascorbic acid did not exhibit any evidence of neural damage, except for the cannula tract. Tissue damage of the SN produced by NMDA was similar to that observed in Experiment 1. Lesions destroyed portions of both pars compacta and pars reticularis of the SN and tended to extend to the parabrachial, minimus, retroenthmoid, and peripeduncular nuclei. Lesion in the SN produced by 6-OHDA affected a similar region of structures to the lesions produced by NMDA. Quantification of SN lesions produced by NMDA and 6-OHDA was determined by cell count. Fig. 7 presents the cell count results in both hemispheres. An overall difference among the three groups was found [$F(2,16) = 23.1$, $P < .001$ for the right hemisphere and $F(2,16) = 58.5$, $P < .001$ for the left hemisphere]. Both NMDA and 6-OHDA reliably decreased the number of SN cells (all Ps $< .001$) and no difference in the number of cells between NMDA and 6-OHDA groups was found. NMDA lesions destroyed about 53% of the SN cell population (51.8% in the right hemisphere and 54.2% in the left hemisphere), whereas 6-OHDA lesions destroyed about 56.8% of SN cells (60.4% in the right hemisphere and 53.2% in the left hemisphere). Therefore, both NMDA and 6-OHDA similarly and effectively destroyed cell bodies in the SN within 24 h after surgery.

3.2.2. HPLC analysis

Dopamine concentration in the striatum and frontal cortex from the three groups is depicted in Fig. 8. In agreement with previous results [41], frontal cortex samples contained very low levels of dopamine in all three groups and no difference among the groups was found [$F(2,16) = 0.7$, $P > .5$]. On the other hand, NMDA lesions in the SN produced a reliable reduction of 34.8% in dopamine levels in the caudate measured 24 h after surgery in comparison with the CONT and 6-OHDA groups (all Ps $< .02$). This result indicates that dopaminergic neurons located at the SN are highly sensitive to NMDA agonist [9,27].

By contrast, 6-OHDA lesions of the SN did not affect the content of dopamine in the caudate. This result appears to indicate that the degenerative effect of 6-OHDA on the dopaminergic neurons in the caudate does not occur during the first 24 h postsurgery. It is known that 6-OHDA effectively destroys dopaminergic neurons [22,39,40]. However, microinjection of 6-OHDA in the SN apparently only leads to a reduction in the nigral dopaminergic terminals during the first week after lesion [20]. Because the present procedure requires that the animals be sacrificed 24 h after lesion, animals infused with 6-OHDA in the SN might apparently not display a reduction of dopamine in the striatum 24 h postlesion. In support of the present finding is the fact that intraventricular injections of 6-OHDA do not alter levels of dopamine in the striatum 24 h after its injection [4,25].

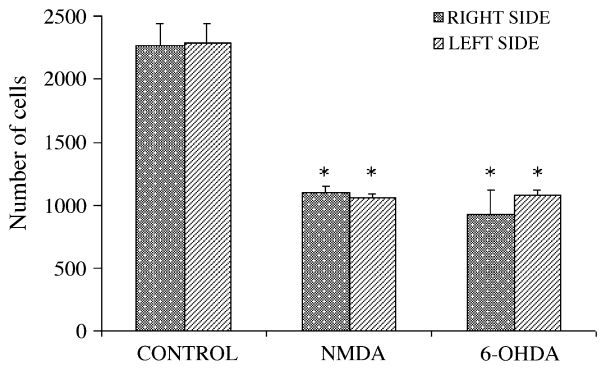

Fig. 7. Mean ± S.E.M. cell count in the left and right SN under high-power magnification 24 h after NMDA (20 μg/μl) or 6-OHDA (8 μg/μl) microinjections into the SN. Control animals consisted of PBS ($n = 3$) or 0.2% of ascorbic acid ($n = 3$) microinjected into the SN. * $P < .001$.

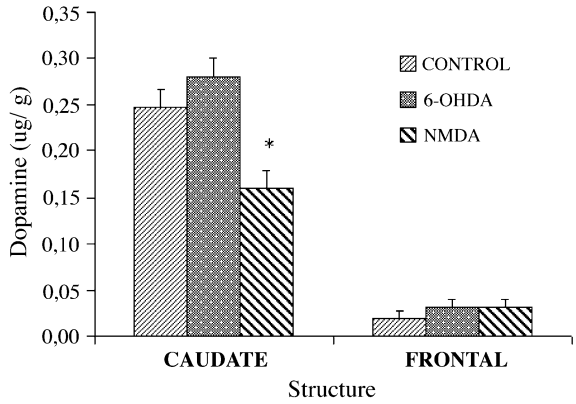

Fig. 8. Mean ± S.E.M. dopamine content in the caudate nucleus and frontal cortex 24 h after NMDA (20 μg/μl) or 6-OHDA (8 μg/μl) microinjections into the SN. Control animals consisted of PBS ($n = 3$) or 0.2% of ascorbic acid ($n = 3$) microinjected into the SN. * $P = .02$ compared with control.

3.2.3. Gastric erosions

Bilateral NMDA lesions of the SN lead to gastric mucosal erosions 24 h after surgery, replicating the findings in Experiment 1 of the present study. On the other hand, lesions in the SN produced by 6-OHDA had no apparent effect on the gastric mucosa. These results are presented in Fig. 9. ANOVA revealed an overall difference among groups [$F(2,16) = 6.62$, $P = .009$]. Pair-wise comparison confirmed that the NMDA group displayed reliably more gastric ulceration than the CONT or 6-OHDA groups (all Ps $< .005$). Absence of a reliable difference between 6-

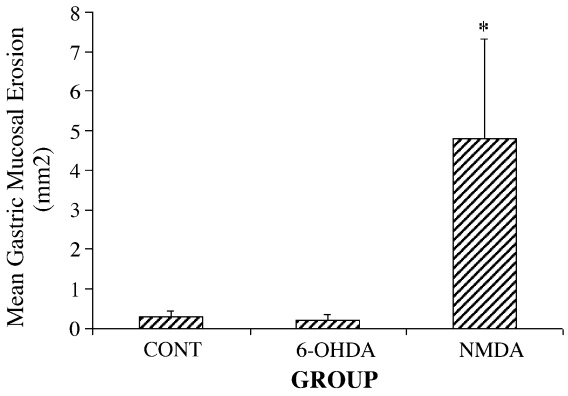

Fig. 9. Mean ± S.E.M. total area of gastric mucosal erosions in rats 24 h after bilateral NMDA (20 μg/μl) or 6-OHDA (8 μg/μl) microinjections into the SN. Control animals consisted of PBS ($n=3$) or 0.2% of ascorbic acid ($n=3$) microinjected into the SN. * P=.005 compared with control.

OHDA and CONT groups indicate that 6-OHDA infusion into the SN did not produce stomach erosions 24 h after surgery.

4. General discussion

The main purpose of the present study was to investigate the participation of dopaminergic systems on stomach erosion formation through the use of NMDA, an excitotoxic agent that selectively destroys cell bodies without affecting fibers of passage. The present results indicate that NMDA lesions of cell bodies in the SN, but not in the VTA, induce gastric erosions within 24 h after surgery. These results replicate a previous report that found the same result pattern using an electrolytic procedure, which not only destroys cell bodies but also disrupts axonal fibers of passage [34]. The present study demonstrates that intrinsic neurons of the SN play an important role for the occurrence of gastric mucosal erosions.

The fact that NMDA lesions of the VTA did not lead to any disruption in the gastric mucosa is in contrast to the findings of Ray et al. [33], who showed that electrolytic lesions of the VTA lead to gastric erosion in nonstressed animals and facilitated gastric erosion formation induced by cold-water restraint. A possible explanation for difference in the present findings and those reported by Ray et al. is that they employed an electrolytic lesioning procedure; thus, the occurrence of gastric erosions may have been due to additional damage to axonal fibers of passage within the VTA area. Roland and Grijalva [34] also observed the presence of gastric erosions 24 h postoperatively in some rats given electrolytic lesions of the VTA. On the other hand, we measured the stomach erosions 24 h after surgery in the present study, whereas Ray et al. examined the stomachs of their animals 2 weeks after the VTA lesions. This suggests that the pathophysiologic processes involved in gastric erosion formation may be time dependent and related to neurodegenerative mechanisms associated with different lesioning techniques.

Destruction of SN cell bodies with the neurotoxic dose of NMDA also leads to a decrease in dopamine levels in the caudate 24 h after surgery, supporting the view that SN dopaminergic neurons may also be involved with gastric mucosal damage. For example, dopamine agonists reduce while dopamine antagonists increase stress-induced gastric lesions when microinjected in the cell body in the SN [14]. Moreover, subcutaneous administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) over 2–3 days produced gastrointestinal ulcers in rats, which could be prevented with the application of dopamine agents [37]. Because MPTP is rapidly metabolized by monoamine oxidase into 1-methyl-4-phenlypyridinium ion (MPP^+) and induces selective destruction of dopaminergic neurons in the pars compacta of the SN, influence of MPTP on gastric function is apparently mediated through the nigrostriatal pathway. Interestingly, it has been reported that NMDA antagonist blocks SN lesions produced by MPP^+ [38], demonstrating the importance of NMDA receptors on SN neurons.

Present results also indicate that although both NMDA and 6-OHDA destroyed the similar amount of cells in the SN, only NMDA lesioning procedure leads to gastric erosions and a reduction in dopaminergic levels in the caudate 24 h after the lesion. These results confirm previous reports, which found that lesions of the SN with 6-OHDA or excitatory amino acids, such as NMDA, might lead to different patterns of results [10,28]. It has been suggested that NMDA and 6-OHDA have different cytotoxic actions in the central nervous system [21]. Increase of intraneuronal 6-OHDA results in the destruction of cellular enzymes and energy-producing cytochromes or related elements of the respiratory chain, which lead the cell to lose its ability to conduct action potentials [22]. On the other hand, NMDA leads to the destruction of the cell by an increase in sodium permeability, which results in a continuous depolarization of the neuron, causing a continuous influx of chloride and other cations, which eventually results in an osmotic lysis [13].

The fact that NMDA neurotoxicity is mediated by the acute excitatory or activational effects, as opposed to 6-OHDA, might indicate that occurrence of gastric ulceration after the infusion of NMDA into the SN is not due to the cell death per se but is related to an overactivation of these cells that precede their death [24,34]. Destruction of SN cell bodies was achieved by either NMDA or 6-OHDA. However, only the microinjection of the neurotoxic dose of NMDA into the SN was able to induce gastric erosions and decrease dopaminergic levels in the caudate. In agreement with this "hyperactivation hypothesis" is the fact that NMDA infusions into the SN increase firing rate and induce burst firing [27]. Finally, axonal transactions of the nigrostriatal tract produced by knife cuts do not lead to erosion formation [18,23],

suggesting that neurons in the SN must be activated before their death to induce the gastric mucosal damage.

Acknowledgements

Research supported by CNPq to J.L.F. (522720-95) and UCLA Academic Senate Research Grant to C.V.G. Thanks are due to Allan Keys for HPLC assistance and Javier Rios-Jimenez for laboratory assistance.

References

[1] Anderson M, Yoshida M. Eletrophysiological evidence for branching nigral projections to the thalamus and superior colliculus. Brain Res 1997;137:361–4.
[2] Beal MF, Kowall NW, Ellison DW, Mazurek MF, Swartz KJ, Martin JB. Replication of the neurochemical characteristics of Huntington's disease by quinolic acid. Nature 1986;321:168–71.
[3] Beckstead RM, Domesick VB, Nauta WJH. Efferent connections of the substantia nigra and ventral tegmental area in the rat. Brain Res 1979;175:197–217.
[4] Bell LJ, Iversen LL, Uretsky NJ. Time course of the effect of 6-hydroxydopamine on catecholamine-containing neurons in rat hypothalamus and striatum. Br J Pharmacol 1970;40:790–9.
[5] Berk ML, Finkelstein JA. Efferent connections of the lateral hypothalamic area of the rat: an autoradiographic investigation. Brain Res Bull 1982;8:511–26.
[6] Bunney BS, Aghajanian GK. The precise localization of nigral afferents in the rat as determined by a retrograde tracing technique. Brain Res 1976;3(117):423–35.
[7] Castro SL, Zigmond MJ. Stress-induced increase in extracellular dopamine in striatum: role of glutamatergic action via *N*-methyl-D-aspartate receptors in substantia nigra. Brain Res 2001;901:47–54.
[8] Childs JA, Gale K. Neurochemical evidence for a nigrotegmental GABAergic projection. Brain Res 1983;258:109–14.
[9] Christoffersen CL, Meltzer LT. Evidence for *N*-methyl-D-aspartate and AMPA subtypes of the glutamate receptor on substantia nigra dopamine neurons: possible preferential role for *N*-methyl-D-aspartate receptors. Neuroscience 1995;67:373–81.
[10] DiChiara G, Olinas M, DelFiacco M, Spano PF, Tagliamonte A. Intranigral kainic acid is evidence that nigral non-dopaminergic neurons control posture. Nature 1977;268:743–5.
[11] DiChiara G, Porceddu ML, Morelli M, Mulas ML, Gessa GL. Evidence for a GABAergic projection from the substantia nigra to the ventromedial thalamus and to the superior colliculus of the rat. Brain Res 1979;176:273–84.
[12] Fallon JH, Loughlin SE. Substantia nigra. In: Paxinos G, editor. The rat nervous system. San Diego (CA): Academic Press; 1995. p. 215–37.
[13] Farber JL, Chien KR, Mittnacht S. Myocardial ischemia: the pathogenesis of irreversible cell injury in ischemia. Am J Pathol 1981;102:271–81.
[14] Glavin GB. Central dopamine involvement in experimental gastrointestinal injury. Prog. Neuro-psychopharmacol. Biol Psychiatry 1992;16:217–21.
[15] Glavin GB. Dopamine: a stress modulator in the brain and gut. Gen Pharmacol 1992;23:1023–6.
[16] Glavin GB. Vulnerability to stress ulcerogenesis in rats differing in anxiety: a dopaminergic correlation. J Physiol 1993;87:239–43.
[17] Glavin GB, Szabo S. Dopamine in gastrointestinal disease. Dig Dis Sci 1990;35:1153–61.
[18] Grijalva CV, Novin D, Bray GA. Alterations in blood glucose, insulin, and free fatty acids following lateral hypothalamic lesions or parasagittal knife cuts. Brain Res Bull 1980;5(Suppl. 4):109–17.
[19] Hernandez DE, Adcock JW, Orlando RC, Patrick KS, Nemeroff CB, Prange AJ. Prevention of stress-induced gastric ulceration by dopamine agonists in the rat. Life Sci 1984;35:2453–8.
[20] Javoy F, Sotelo C, Herbert A, Agid Y. Specificity of dopaminergic neuronal degeneration induced by intracerebral injection of 6-hydroxy dopamine in the nigrostriatal dopamine system. Brain Res 1976;102:201–15.
[21] Jonsson G. Chemical neurotoxins as denervation tools in neurobiology. Annu Rev Neurosci 1980;3:169–87.
[22] Kostrzewa RW, Acobowitz DM. Pharmacological actions of 6-hydroxydopamine. Pharmacol Rev 1974;26:199–288.
[23] Landeira-Fernandez J, Grijalva CV. Gastric mucosal erosion produced by NMDA microinfusions in the lateral hypothalamus: effects of selective knife cuts. Behav Brain Res 1999;102:51–60.
[24] Landeira-Fernandez J, Grijalva CV. Infusions of neurotoxic doses of *N*-methyl-D-aspartate (NMDA) into the lateral hypothalamus: acute effects on gastric erosion formation, temperature regulation and feeding behavior. Behav Neurosci 1999;113:1049–61.
[25] Laverty R, Taylor KM. Effects of intraventricular 2,4,5-trihydroxydopamine (6-hydroxydopamine) on rat behavior and brain catecholamine metabolism. Br J Pharmacol 1970;40:836–46.
[26] Marshall JF, Richardson JS, Teitelbaum P. Nigrostriatal bundle damage and the lateral hypothalamic syndrome. J Comp Physiol Psychol 1974;3:375–95.
[27] Meltzer LT, Christoffersen CL, Serpa KA. Modulation of dopamine neuronal activity by glutamate receptors subtypes. Neurosci Biobehav Rev 1997;21:511–8.
[28] Nitsch C, Wolfrum G, Schaefer F, Scotti AL, Unger J. Opposite effects of intranigral ibotenic acid and 6-hydroxydopamine on motor behavior and on striatal neuropeptide Y neurons. Brain Res Bull 1992;30:21–32.
[29] Nomura K, Maeda N, Yoshino T, Yamaguchi I. Different mechanisms mediated by dopamine D1 and D2 receptors are involved etiologically in activity-stress gastric lesion of the rat. J Pharmacol Exp Ther 1995;273:1001–7.
[30] Ottersen OP, Storm-Mathisen J. Glutamate- and GABA-containing neurons in the mouse and rat brain, as demonstrated with a new immunocytochemical technique. J Comp Neurol 1984;229:374–92.
[31] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1986.
[32] Puri S, Ray A, Chakravarti AK, Sen P. Role of dopaminergic mechanisms in the regulation of stress responses in experimental animals. Pharmacol Biochem Behav 1994;48:53–6.
[33] Ray A, Henke PG, Sullivan RM. Central dopamine systems and gastric stress pathology in rats. Physiol Behav 1988;42:359–64.
[34] Roland BL, Grijalva CV. Gastric mucosal erosions induced by lateral hypothalamic damage: neuronal and dopaminergic mechanisms. Brain Res 1993;605:110–20.
[35] Sullivan RM, Szechtman H. Asymmetrical influence of mesocortical dopamine depletion on stress ulcer development and subcortical dopamine systems in rats: implications for psychopathology. Neuroscience 1995;65:757–66.
[36] Swanson LW. The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res Bull 1982;9:321–53.
[37] Szabo S, Brown A, Pihan G, Dali H, Neumeyer JL. Duodenal ulcer induced by MPTP (1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine). Proc Soc Exp Biol Med 1985;180:567–71.
[38] Turski L, Bressler K, Rettig KJ, Loschmann PA, Wachtel H. Protection of substantia nigra from MPP+ neurotoxicity by *N*-methyl-D-aspartate antagonists. Nature 1991;349:414–8.
[39] Ungerstedt U. Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol Scand, Suppl 1971;367:1–48.
[40] Uretsky NJ, Iveresen LL. Effects of 6-hydroxydopamine on catecho-

lamine containing neurons in the rat brain. J Neurochem 1970;17: 269–78.
[41] Vicent SR, Hattori T, McGeer EG. The nigrotectal projection: a biochemical and ultrasonic characterization. Brain Res 1978;151: 159–64.
[42] Winn P, Tarbuck A, Dunnett SB. Ibotenic acid lesions of the lateral hypothalamus: comparison with the electrolytic lesion syndrome. Neuroscience 1984;12:225–40.
[43] Young AB, Dauth GW, Hollingsworth Z, Penney JB, Kaatz K, Gilman S. Quisqualate and NMDA-sensitive ^{3}H glutamate binding in primate brain. J Neurosci Res 1990;27:512–21.