Riluzole Blocks Dopamine Release Evoked by N-methyl-D-aspartate, Kainate, and Veratridine in the Rat Striatum

Hawa Keita, M.D.,* Claire Lepouse, M.D.,* Danielle Henzel,† Jean-Marie Desmonts, M.D.,‡ and Jean Mantz, M.D., Ph.D.§

Background: Dopamine (DA) is released in large amounts during cerebral ischemia and may exacerbate tissue damage. Riluzole (54274 RP) is a recently developed agent that depresses glutamate neurotransmission in the central nervous system (CNS) and that may protect against ischemic injury in some animal models. Because glutamate stimulates the release of DA in the striatum, the authors hypothesized that riluzole could antagonize DA release in this structure.

Methods: Assay for DA release consisted of superfusing ^{3}H-DA preloaded synaptosomes with artificial cerebrospinal fluid (1 ml/min, 37°C) and measuring the radioactivity obtained from 1-min fractions over 22 min, first in the absence of any treatment (spontaneous release, 8 min), then in the presence of depolarizing agents combined with riluzole (0.1–100 μM, 5 min), and finally with no pharmacologic stimulation (9 min). The following depolarizing agents were tested: KCl (9, 15 mM), veratridine (0.01–1 μM), N-methyl-D-aspartate (NMDA, 0.1–1 mM), kainate (0.1–1 mM), and nicotine (0.01–0.5 mM). Assay for DA uptake was performed by measuring the radioactivity incorporated in synaptosomes incubated with ^{3}H-DA (44 nM; 5 min; 37°C).

Results: All depolarizing agents produced a significant, concentration-related increase from basal ^{3}H-DA release. Riluzole was found to decrease the release induced by veratridine (1 μM), NMDA (1 mM), and kainate (1 mM) in a significant, concentration-related manner (IC$_{50}$ = 9.5 μM, 1.6 μM, and 5.8 μM for veratridine, NMDA, and kainate, respectively). In contrast, it did not affect the release elicited by either KCl or nicotine. Riluzole had no significant effect on the specific ^{3}H-DA uptake.

Conclusions: Riluzole produced a potent blockade of the release of DA mediated by activation of presynaptic sodium channels, NMDA, and kainate receptors. Depression of glutamate transmission together with blockade of DA release may contribute to the actions of this agent *in vivo.* (Key words: Animals: rats. Brain: ischemia; striatal; striatum. Pharmacology: AMPA receptors; depressant; dopamine; NMDA receptors; nicotinic receptors; riluzole; voltage-operated calcium channels; voltage-operated sodium channels. Synapse: presynaptic; release; uptake.)

CEREBRAL ischemia causes axon terminals to release excitotoxic glutamate and open N-methyl-D-aspartate (NMDA) channels, which allow Ca^{2+} entry into the neurons and trigger a cascade of intracellular events leading to neuronal damage.[1-3] Riluzole (2-amino-6-trifluoro-methoxy benzothiazole, 54274 RP, PK 26124)[4] is a recently developed agent that exhibits anesthetic properties and that may protect against ischemic neuronal death in some animal models.[5-7] It has been shown to depress glutamate release at mammalian central nervous system (CNS) synapses.[8] Although its mechanism of action has not been clearly established, depressant actions on the conduction of presynaptic glutamate fibers,[9] sodium channels,[10] or NMDA-induced Ca^{2+} currents[6,11] have been reported.

In the rat striatum, a functionally important supraspinal brain area, glutamate terminals originating from the cerebral cortex make synaptic contact with DA nerve endings arising from the nigrostriatal pathway.[12] Mimicking activation of the corticostriatal glutamate fibers by local application of glutamate agonists in this brain area results in a potent stimulation of dopamine (DA) release mediated by NMDA and AMPA receptors located on the presynaptic DA terminals.[13-16] Alternatively, DA may be released by activation of voltage-operated Ca^{2+} (VOCC) or sodium channels (VOSC) or nicotinic recep-

Fig. 1. Schematic representation of the presynaptic receptors or ionic channels present on the striatal DA terminals. NMDA and kainate receptors are stimulated by glutamate released presynaptically from corticostriatal glutamatergic inputs, whereas nicotinic receptors are activated by acetylcholine originating from striatal interneurons. Alternatively, DA released can be elicited by activation of the voltage-operated sodium channels achieved by veratridine or the voltage-operated calcium channels stimulated by KCl. All depolarizing stimuli or natural depolarization of the nerve terminal achieved by action potential propagation lead to an increase in intracellular concentrations and subsequent DA release into the synaptic cleft. NMDA-r = N-methyl-D-aspartate receptor; kainate-r = kainate receptor; nicotinic-r = nicotinic receptor; VOSC = voltage-operated sodium channels; VOCC = voltage-operated Ca^{2+} channels; Ca^{2+}_i = intracellular calcium.

tors present on the DA terminals (fig. 1).[17] Because DA is released in large amounts into the striatum during cerebral ischemia and may exacerbate tissue damage,[18,19] we hypothesized that riluzole may influence the release or the high affinity uptake of this transmitter.

Materials and Methods

Handling procedures, as written in the *Guide for the Care and Use of Laboratory Animals*‖ were followed throughout. Experiments were performed on male Sprague-Dawley rats (Iffa-Credo, L'Arbresle, France) weighing 200–225 g and housed on a 12:12 light/dark cycle with food and water *ad libitum*. Approval was obtained from the Institutional Animal Care and Use Committee at the University of Paris 7.

‖ Guide for the Care and Use of Laboratory Animals, Health and Human Services, NIH Publication N° 86-23, Revised 1996, pp 58–9.

Preparation of Striatal Synaptosomes

Animals were killed by stunning and decapitation. After longitudinal hemisection of the brain, striata were quickly removed and processed to a purified synaptosomal preparation as previously reported in detail.[17,20] Synaptosomes were diluted up to 0.16 mg/ml in an ice-cold artificial cerebrospinal fluid (CSF) containing (in mM): NaCl, 126.5; $NaHCO_3$, 27.5; KCl, 1; KH_2PO_4, 0.5; $CaCl_2$, 1.1; $MgCl_2$, 0.83; Na_2SO_4, 0.5; glucose, 5.9; pargyline, 10^{-2}; ascorbic acid, 1, adjusted at pH 7.3 with 95%/5% [v/v] $O_2 - CO_2$ mixture. In Ca^{2+} (Mg^{2+}, respectively)-free experiments, $CaCl_2$ ($MgCl_2$, respectively) was omitted from the CSF.

Dopamine Release Assay

Uptake of DA was performed by incubating (10 min; 37°C) the synaptosomes with $[2,5,6]^3$H-DA (Amersham, Little Chalfont, UK; > 44 nmol/l, 24 Ci/mmol, 1 mCi/ml). Aliquots (1 ml) were pipetted into each of the superfusion chambers (0.3 ml volume each) and embedded in Whatman GF/F glass filters (0.70-μm retention capacity) by light suction, then superfused at a 1 ml/min flow rate using a superfusion device equipped with an automatic fraction collector (Brandel, Gaithersburg, MD). The 1 ml/min flow rate was selected because of its ability to prevent reuptake of ^{3}H-DA by the synaptosome preparation.[18,20] After a 30-min washing step (37°C), serial fractions were collected every minute during three consecutive periods: the mean value of the radioactivity (counts/min measured over the first 8 min by liquid scintillation spectrometry using Aquasol-2 (New England Nuclear, Boston, MA) was considered the basal (spontaneous) ^{3}H-DA release. During the next 5 min of superfusion, either no treatment (time-dependent control) or various pharmacologic agents were delivered to the preparation, and radioactivity again was estimated in each fraction. Because of a 3-ml dead volume occupied by the inside of the connectubes, pharmacologic agents delivered at a 1-ml/min flow rate reached synaptosomes only after a delay, which averaged 180 s. For the final 9 min, radioactivity was determined from 1-min fractions in the absence of any treatment, although in the experiments on the nicotine-induced release, fractions were collected every 30 s because of the rapid desensitization of the nicotinic response.[21] The influence of a drug on the release of ^{3}H-DA was assessed by calculating the difference between the release observed in the presence and absence (time-dependent control) of the pharmacologic agent

used. This was expressed as a fractional increase from spontaneous release.

To allow comparison with previous studies, the concentrations of the pharmacologic agents tested were: KCl (9, 15 mM), NMDA (50 μM, 100 μM, 500 μM, and 1 mM in a Mg^{2+}-free superfusion medium, Sigma, L'Isle d'Abeau, France), kainate (50 μM, 100 μM, 500 μM, and 1 mM, Sigma), the noncompetitive NMDA receptor antagonist MK801 (100 μM, Merck-Sharp and Dohme, Darmstadt, Germany), the AMPA/kainate receptor antagonist 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX, 100 μM, Research Biochemical Incorporated, Illkirch, France), nicotine (10, 50, 100, and 500 μM, Sigma), the nicotinic receptor antagonist mecamylamine (10 μM, Sigma), veratridine (0.01, 0.1, 0.5, and 1 μM, Sigma), and the blocker of the VOSC tetrodotoxin (TTX, 0.1 μM, Sigma). In these experiments, mecamylamine, MK801, CNQX, and TTX were coapplied together with nicotine (0.5 mM), NMDA (1 mM), kainate (1 mM), and veratridine (1 μM), respectively. In addition, to further assess the role played by the VOSC, the influence of TTX (0.1 μM) on the release elicited by nicotine, NMDA, and kainate was also examined. Riluzole was dissolved in dimethylsulphoxide (Merck-Sharp and Dohme) and tested over a 10^{-7} – 10^{-4} M concentration range.

Dopamine Uptake Assay

Dopamine uptake was performed as previously described.[22,23] Briefly, ^{3}H-DA was added to the synaptosomal suspension at 37°C in either the absence of any pharmacologic agent (control) or in the presence of various concentrations of nomifensine (10^{-8} – 5×10^{-5} M, Research Biochemical Incorporated) or riluzole (10^{-7} – 10^{-4} M). After 5-min incubation, the mixture was vacuum-filtered through Whatman GF/F filters and washed with 30 ml of ice-cold CSF. This duration of incubation was determined according to preliminary experiments, indicating that ^{3}H-DA increased linearly and reproducibly with time between 3 and 7 min. The radioactivity retained on the filters was extracted with NaOH 0.1 M and measured by liquid scintillation. Specific ^{3}H-DA uptake was considered the difference between the radioactivity measured in the presence and absence of nomifensine 10^{-5} M.

Data Analysis

Results (mean ± SEM) were considered reliable only if they had been reproduced in four independent experiments (each of them performed in triplicate). Concentration – response curves, IC_{50} values, and Hill coefficients
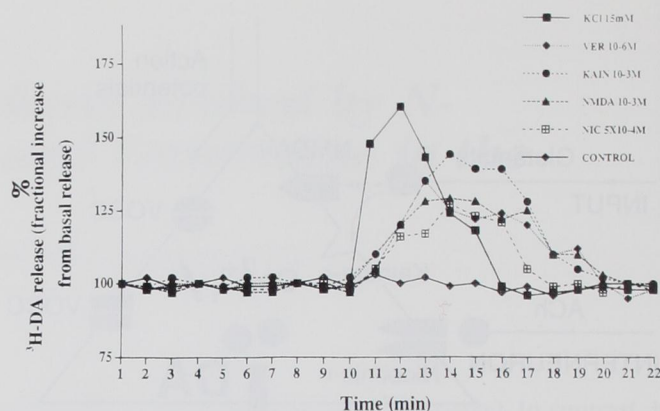

Fig. 2. Typical example of the time-course of the release rate of ^{3}H-DA elicited by various depolarizing agents from preloaded striatal synaptosomes. Data are expressed as a fractional increase from basal release. Depolarizing agents were applied between minutes 8 and 13 (see Methods section).

cients were generated directly from computer using the GraphPAD software (Intuitive Software for Science, San Diego, CA). The function used to fit the curves to the data was the following four-parameter logistic equation:

$$Y = A + (B - A)/[1 + (10^C/10^X)^D]$$

where X represents the logarithm of riluzole concentration, A and B, respectively, the minimum (bottom) and maximum (top) of X values. C is the logarithm of the IC_{50} value, and D is the Hill coefficient or slope factor that is positive for curves in which the Y value increases with increasing X and is negative for curves in which the Y value decreases with increasing X. Statistics were performed by analysis of variance (ANOVA) followed by the Student's t test corrected for the number of comparisons. A P value less than 0.05 was considered the threshold for significance.

Results

Pharmacologic Characterization of the Depolarization-evoked ^{3}H-dopamine Release from Striatal Synaptosomes

The ^{3}H-DA concentration estimated in the incubation medium during the uptake phase was 250 ± 15 pmol/mg protein, and the initial basal release rate measured over 5 min was approximately 1.04 ± 0.06 pmol · mg protein^{-1} · min^{-1}. Basal release rate was not affected by removing Ca^{2+} or Mg^{2+} from the superfusion medium. A typical example of the time-course of the ^{3}H-DA release observed with the different paradigms used is displayed in figure 2.
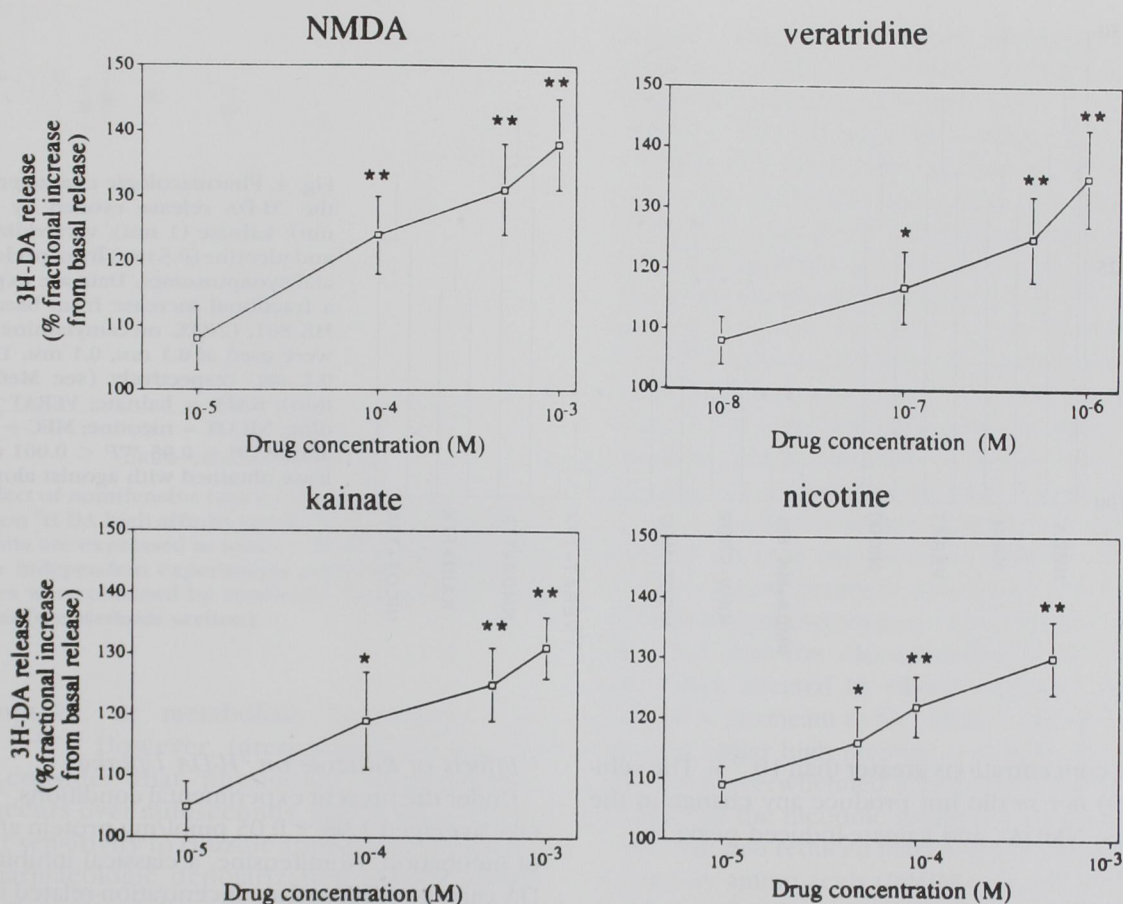
Fig. 3. Dose–response curves for NMDA (*upper left*), kainate (*lower left*), veratridine (*upper right*) and nicotine (*lower right*) for ³H-DA release from preloaded striatal synapyosomes. Data (mean ± SEM) are expressed as a fractional increase from basal release. $^*P < 0.05$. $^{**}P < 0.01$ *versus* basal release.

As displayed in figure 3, all depolarizing agents elicited a significant, concentration-related increase from spontaneous ³H-DA release. The ³H-DA peak elicited by KCl (9 and 15 mM) was (in fractional units): 1.32 ± 0.06 ($P < 0.01$) and 1.61 ± 0.08 ($P < 0.01$), respectively. It was followed by a rapid return to baseline after cessation of application of the pharmacologic agent. In contrast, the recovery of basal release rate was slower after veratridine, NMDA, kainate, and nicotine application. The effect of KCl was markedly reduced when Ca^{2+} was removed from the external medium ($-87 \pm 8\%$). The effects of NMDA, kainate, nicotine, and veratridine, respectively, on ³H-DA release were markedly reduced by MK 801 ($-96 \pm 6\%$), CNQX ($-89 \pm 9\%$), mecamylamine ($-85 \pm 11\%$), and TTX ($-97 \pm 5\%$), respectively. Application of TTX did not significantly affect the NMDA-, or nicotine-stimulated release, although it

slightly, but significantly, attenuated the kainate-induced DA release (fig. 4).

Effects of Riluzole on ³H-DA Release

Riluzole caused a significant, concentration-related, decrease in the release of ³H-DA evoked by NMDA, kainate, and veratridine (fig. 5). The best fit of the curves to the data was always obtained when Hill coefficient was forced to 1. The IC_{50} values for the inhibitory effect of riluzole were 9.5 μM, 1.6 μM, and 5.8 μM, respectively, for the veratridine-, NMDA-, and kainate-induced, respectively, ³H-DA release. In contrast, riluzole failed to significantly alter the release elicited by either KCl (9 and 15 mM) or nicotine (0.5 mM). The attenuation of the NMDA (kainate, respectively)-evoked peaks of radioactivity by riluzole yielded a ceiling effect as 36% (40%, respectively) of the release was unaffected
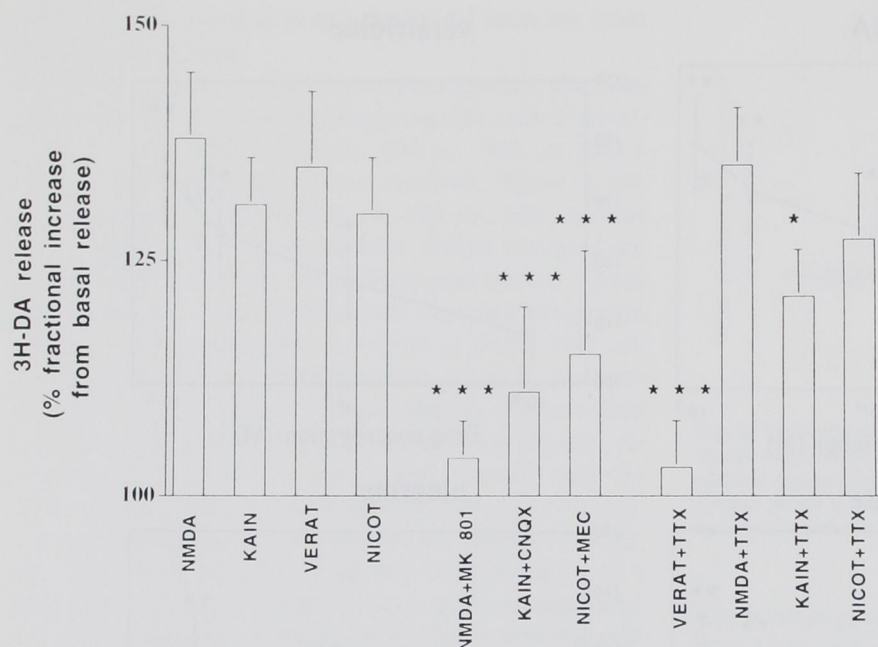
Fig. 4. Pharmacologic characterization of the ^{3}H-DA release evoked by NMDA (1 mM), kainate (1 mM), veratridine (1 µM), and nicotine (0.5 mM) from preloaded striatal synaptosomes. Data are expressed as a fractional increase from basal release. MK-801, CNQX, mecamylamine, and TTX were used at 0.1 mM, 0.1 mM, 10 µM, and 0.1 µM, respectively (see Methods section). KAIN = kainate; VERAT = veratridine; NICOT = nicotine; MEC = mecamylamine. *$P < 0.05$ ***$P < 0.001$ *versus* release obtained with agonist alone.

by riluzole concentrations greater than 10^{-5} M. The vehicle (DMSO) *per se* did not produce any change in the veratridine-, NMDA-, and kainate-induced peaks.
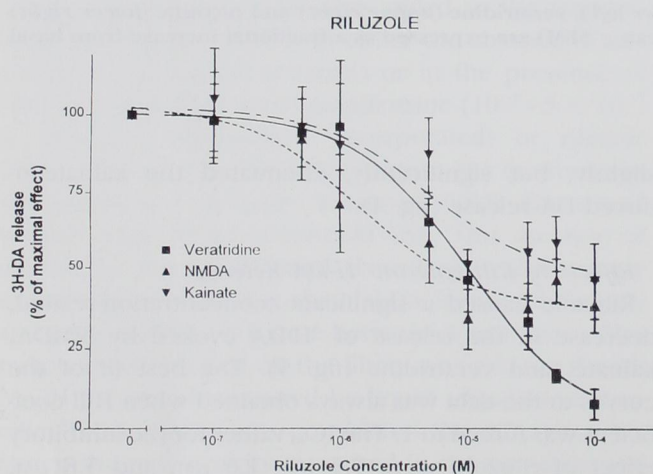

Fig. 5. Concentration–response curves for riluzole effect on the depolarization-evoked ^{3}H-DA release mediated by veratridine (1 µM), NMDA (1 mM), and kainate (1 mM) on striatal synaptosomes. Data are expressed as mean ± SEM and were obtained from at least four independent experiments performed in triplicate. For veratridine-, NMDA-, and kainate-induced release, Hill coefficients and IC_{50} values could be derived from nonlinear computerized curve fitting of the data (see Methods section).

Effects of Riluzole on ^{3}H-DA Uptake

Under the present experimental conditions, ^{3}H-DA uptake averaged 1.98 ± 0.05 pmol/mg protein after 5 min of incubation. Nomifensine, a classical inhibitor of the DA carrier, produced a concentration-related inhibition of ^{3}H-DA uptake, the IC_{50} value being 0.24 µM. In contrast, riluzole failed to affect ^{3}H-DA uptake at any of the concentrations tested (fig. 6).

Discussion

The present study indicates that riluzole is able to block the depolarization-evoked release of DA from striatal nerve terminals, although the ability of riluzole to reduce DA release depends on the nature of the depolarizing stimulus; it markedly decreases the veratridine-elicited release mediated by activation of presynaptic VOSC. It also exhibits a significant inhibitory effect on presynaptic events mediated by ionotropic glutamate receptors because it potently reduces the NMDA- and kainate-evoked DA release. In contrast, riluzole fails to affect the release elicited by activation of the presynaptic nicotinic receptors or involving the VOCC (KCl-evoked release).

Synaptosomes provide a reliable and reproducible model for analyzing, *in vitro*, the direct presynaptic effects of pharmacologic agents on neurotransmitter re-
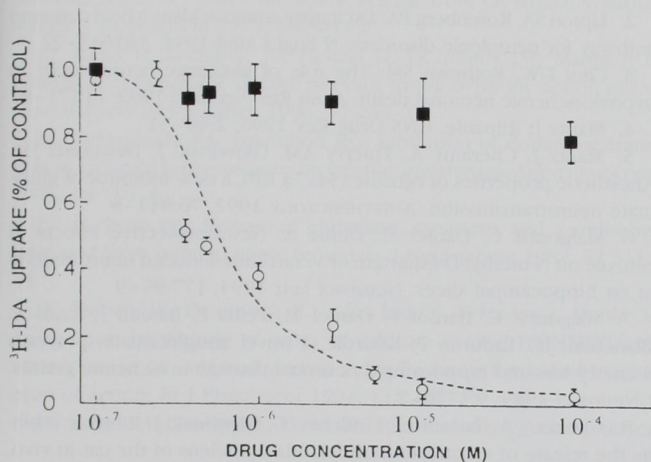
Fig. 6. Effect of nomifensine (open circles) and riluzole (filled squares) on ^{3}H-DA high affinity uptake by rat striatal synaptosomes. Data are expressed as mean ± SEM and result from at least four independent experiments performed in triplicate. IC$_{50}$ values were obtained by nonlinear computerized fitting of the data (see Methods section).

lease, uptake, or metabolism in various brain areas.[13–17,21–24] However, interpretation of the data should consider that physiologic depolarization, which occurs over milliseconds, may have exhibited different sensitivity to riluzole application compared with pharmacologic depolarization applied during several minutes to the synaptosomal preparation. Also, it cannot be excluded that DA nerve terminals, *in situ,* subjected to impulse activity and a transmitter-enriched environment may behave differently.

The present results show that riluzole blocks the release of DA elicited by activation of the VOSC. DA release was stimulated by veratridine, an activator of sodium channels, and blocked by TTX application. These data are consistent with previous results indicating that riluzole (10 μM) reduced the increase in intracellular Ca^{2+} concentration evoked by veratridine (10 μM) on cultured granule cells.[25] Further, riluzole (1–100 μM) has been shown to reduce the magnitude of the peak sodium currents carried by the cloned rat brain IIA sodium channel α-subunits expressed in *Xenopus* oocytes.[10] Looking at the mechanisms of riluzole action on sodium channels, several lines of evidence suggest that riluzole may increase inactivation at all potentials by high affinity block of inactivated channels.[10] Whether this may occur at DA presynaptic nerve endings cannot be inferred from our data. However, this action reported on DA fibers may also apply to other subpopulations of nerve endings, such as the gluta-

matergic terminals, for which riluzole causes potent depression of the nerve conduction that resembles that produced by some local anesthetics.[9]

Riluzole did not affect the release of DA stimulated by either KCl or nicotine application to the synaptosomes. The KCl-evoked peak was not observed in the absence of external Ca^{2+}, whereas the nicotine-induced effect was abolished in the presence of mecamylamine, a nicotinic antagonist exhibiting particular efficiency in blocking nicotinic receptors of the striatal DA terminals.[26] The absence of effect of riluzole on the synaptosomal DA release coupled to activation of presynaptic VOCC or nicotinic receptor-coupled channels suggests that these specific channels are not sensitive to riluzole. although we cannot rule out that riluzole could act at any of a number of sites to depress DA release in the striatum because the process of release is known to involve several proteins associated with vesicle and nerve terminal membranes. Alternatively, it could be expected that the nicotine-induced DA release may have been affected by riluzole because the nicotinic channel is permeant to Na$^+$ ions. It cannot be excluded that the rather high nicotine concentrations used here may have overwhelmed a potential blocking effect of riluzole on the nicotinic receptor-coupled channel.

Riluzole also reduced markedly DA release evoked by excitatory amino acids (NMDA and kainate). The peak elicited by NMDA application was blocked by application of the NMDA receptor antagonist MK 801, but not TTX, whereas that produced by kainate was blocked by the AMPA receptor antagonist CNQX and slightly, but significantly, attenuated by TTX. This indicates that this amino acid-stimulated release was a result of activation of the NMDA and AMPA receptors present on the presynaptic DA terminals and that VOSCs are not significantly involved in NMDA-evoked DA release, which is consistent with previous findings.[25] Thus, riluzole also depresses amino acid-mediated transmission by blocking responses involving glutamate ionotropic receptors located on presynaptic DA terminals. Riluzole has been shown to antagonize NMDA receptor-mediated currents in *Xenopus* oocytes injected with mRNA from rat whole brain cortex, although the effect is less potent (IC$_{50}$ = 18.2 μM).[11] It had only a weak blocking effect (IC$_{50}$ = 167 μM) on the kainate-mediated responses in this model. The low IC$_{50}$ values reported in our study suggest that the responses mediated by presynaptic NMDA and kainate receptors located on DA nerve endings may be particularly sensitive to riluzole.

It is not clear how riluzole blocks electrophysiologic

responses mediated by excitatory amino acids because no interaction between this agent and any of the recognition sites on the NMDA or AMPA receptors has been demonstrated.[4] Also, riluzole did not interact with either the glycine site or the phencyclidine site of the NMDA ionotropic receptor and did not block metabotropic receptors coupled to phospholipase C.[4,27,28] Recently, Hubert *et al.* have shown that riluzole antagonized the NMDA-induced increase in intracellular Ca^{2+} *via* a pertussis toxin sensitive mechanism.[25] This supports that riluzole can act at G proteins located in the immediate environment of the NMDA receptor itself. The NMDA- and kainate-stimulated DA release could be antagonized only in part by riluzole because a ceiling effect was observed at concentrations greater than 10 μM. Altogether, these data suggest that riluzole blocks presynaptic ionotropic receptors *via* either direct, noncompetitive, or, more likely, by indirect mechanisms.

The presence of excessive concentrations of glutamate in the synaptic cleft has been involved in the so-called excitotoxic process in the CNS. This is favored by certain conditions including a failure of glutamate uptake, prolonged and excessive depolarization of the nerve terminal, or introduction of glutamic acid from a nonregulated source.[1-3] It has been shown that riluzole decreases glutamate transmission by acting at presynaptic target sites, including conduction velocity and neurotransmitter release.[4,8,9] Our results indicate that, in addition to these previously reported actions, riluzole blocks the responses mediated by the presynaptic VOSC, NMDA, and kainate receptors located on the striatal DA terminals. The fact that riluzole blocks excitatory amino acid-mediated neurotransmission by acting at pre- and postsynaptic target sites and, on the other hand, antagonizes the activity of the VOSC is consistent with one possible mechanism of antiischemic activity.

In conclusion, we provide evidence that riluzole exerts a selective depression of the release of DA elicited by excitatory amino acids and veratridine in the rat striatum *in vitro*. Interestingly, several lines of evidence indicate that the striatal DA is released in large quantities into the extracellular space during cerebral ischemia and may exacerbate tissue damage.[18,19,29,30] Depression of glutamate transmission together with blockade of DA release may provide a pharmacologic basis to better understand some neuroprotective effects of riluzole *in vivo*.

References

1. Meldrum B, Garthwaite J: Excitatory amino acid neurotoxicity and neurodegenerative disease. Trends Pharmacol Sci 1990; 11:379–87

2. Lipton SA, Rosenberg PA: Excitatory amino acids as a final common pathway for neurologic disorders. N Engl J Med 1994; 330:613–22

3. Choi DW, Rothman SM: The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. Annu Rev Neurosci 1990; 13:171–82

4. Mantz J: Riluzole. CNS Drug Rev 1996; 2:40–51

5. Mantz J, Cheramy A, Thierry AM, Glowinski J, Desmonts JM: Anesthetic properties of riluzole (54274 RP), a new inhibitor of glutamate neurotransmission. ANESTHESIOLOGY 1992; 76:844–8

6. Malgouris C, Daniel M, Doble A: Neuroprotective effects of riluzole on N-methyl-D-aspartate or veratridine-induced neurotoxicity in rat hippocampal slices. Neurosci Lett 1994; 177:95–9

7. Malgouris C, Bardot F, Daniel M, Pellis F, Rataud J, Uzan A, Blanchard JC, Laduron P: Riluzole, a novel antiglutamate, prevents memory loss and hippocampal neuronal damage in ischemic gerbils. J Neurosci 1989; 9:3720–7

8. Cheramy A, Barbeito L, Godeheu G, Glowinski J: Riluzole inhibits the release of glutamate in the caudate nucleus of the cat in vivo. Neurosci Lett 1992; 147:209–12

9. MacIver MB, Amagasu SM, Mikulec AA, Monroe FA: Riluzole anesthesia: Use-dependent block of presynaptic glutamate fibers. ANESTHESIOLOGY 1996; 85:626–34

10. Hebert T, Drapeau P, Pradier L, Dunn RJ: Block of the rat brain IIA sodium channel α subunit by the neuroprotective drug riluzole. Mol Pharmacol 1994; 45:1055–60

11. Debono MW, Le Guern J, Canton T, Doble A, Pradier L: Inhibition by riluzole of electrophysiological responses mediated by rat kainate and NMDA receptors expressed in Xenopus oocytes. Eur J Pharmacol 1993; 235:283–9

12. Graybiel AM: Neurotransmitters and neuromodulators in the basal ganglia. Trends Neurosci 1990; 13:244–54

13. Desce JM, Godeheu G, Galli T, Artaud F, Cheramy A, Glowinski J: Presynaptic facilitation of dopamine release through D,L-α-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptors on synaptosomes from the rat striatum. J Pharmacol Exp Ther 1991; 259:692–8

14. Desce JM, Godeheu G, Galli T, Arataud F, Cheramy A, Glowinski J: L-glutamate — evoked release of dopamine from synaptosomes of the rat striatum: involvement of AMPA and N-methyl-D-aspartate receptors. Neuroscience 1992; 47:333–9

15. Krebs MO, Desce JM, Kemel ML, Gauchy C, Godeheu G, Cheramy A, Glowinski J: Glutamatergic control of dopamine release in the rat striatum: evidence for presynaptic N-methyl-D-aspartate receptors on dopaminergic nerve terminals. J Neurochem 1991; 56:81–5

16. Wang JKT: Presynaptic glutamate receptors modulate dopamine release from striatal synaptosomes. J Neurochem 1991; 57:819–22

17. Mantz J, Varlet C, Lecharny JB, Henzel D, Lenot P, Desmonts JM: Effects of volatile anesthetics, thiopental, and ketamine on spontaneous and depolarization-evoked dopamine release from striatal synaptosomes in the rat. ANESTHESIOLOGY 1994; 80:352–63

18. Kahn RA, Weinberger J, Brannan T, Prikhojan A, Reich DL: Nitric oxide modulates dopamine release during global temporary cerebral ischemia. Anesth Analg 1995; 80:1116–21

19. Globus MY-T, Gonsberg MD, Dietrich WD, Busto R, Scheinberg P: Substantia nigra lesion protects against ischemic damage in the striatum. Neurosci Lett 1987; 80:251–6

20. Dunkley PR, Heath JW, Harrison SM, Jarvie PE, Glenfield PJ, Rostas JAP: A rapid Percoll gradient procedure for isolation of synaptosomes directly from an S1 fraction: Homogeneity and morphology of subcellular fractions. Brain Res 1988; 441:59–71

21. Rowell PP, Hillebrand JA: Characterization of nicotine-induced

desensitization of evoked dopamine release from rat striatal synapto-somes. J Neurochem 1994; 63:561–9

22. Keita H, Lecharny JB, Henzel D, Desmonts JM, Mantz J: Is inhibition of dopamine uptake relevant to the hypnotic action of i.v. anaesthetics? Br J Anaesth 1996; 77:254–6

23. El-Maghrabi EA, Eckenhoff RG: Inhibition of dopamine transport in rat brain synaptosomes by volatile anesthetics. ANESTHESIOLOGY 1993; 78:750–6

24. Nicholls DG: Release of glutamate, aspartate, and γ-aminobu-tyric acid from isolated nerve terminals. J Neurochem 1989; 52:331–41

25. Hubert JP, Delumeau JC, Glowinski J, Prémont J, Doble A: Antagonism by riluzole of entry of calcium evoked by NMDA and veratridine in rat cultured granule cells: Evidence for a dual mecha-nism of action. Br J Pharmacol 1994; 113:261–7

26. Sacaan AI, Dunlop JL, Lloyd GK: Pharmacological characteriza-tion of neuronal acetylcholine gated ion channel receptor-mediated hippocampal norepinephrine and striatal dopamine release from rat brain slices. J Pharmacol Exp Ther 1995; 274:224–30

27. Benavides J, Camelin JC, Mitrani N, Flamand F, Uzan A, Legrand JJ, Guérémy, Le Fur G: 2-Amino-6-trifluoromethoxy benzothiazole, a possible antagonist of excitatory aminocid neurotransmission. II. Biochemical properties. Neuropharmacology 1985; 24:1085–92

28. Doble A, Perrier ML: Pharmacology of excitatory amino acid receptors coupled to inositol phosphate metabolism in neonatal stria-tum. Neurochem Int 1989; 15:1–9

29. Phebus LA, Clemens JA: Effects of transient, global, cerebral ischemia on striatal extracellular dopamine, serotonin and their me-tabolites. Life Sci 1989; 44:1135–42

30. Globus MY-T, Busto R, Dietrich WD, Martinez E, Valdes I, Ginsberg MD: Intraischemic extracellular release of dopamine and glutamate is associated with striatal vulnerability to ischemia. Neu-rosci Lett 1988; 91:36–40