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Trialkylglycines: A New Family of Compounds with *in Vivo* Neuroprotective Activity

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ABSTRACT

Glutamate neurotoxicity is involved in the pathogenesis of neurodegenerative disorders such as Huntington's, Parkinson's and Alzheimer's diseases. It plays also a major role in the neuronal damage that occurs in brain ischemia and head trauma. Finding molecules that prevent or reverse glutamate neurotoxicity (excitotoxicity) is, therefore, of great interest. Strategies aimed at this end include the screening of libraries of compounds synthesized by combinatorial chemistry to find molecules that prevent neuronal death *in vitro* and *in vivo*. A library of trialkylglycines was screened to assess whether they prevent glutamate-induced neuronal death in primary cultures of cerebellar neurons. Two types of trialkylglycines have been found that significantly reduce the incidence of glutamate-induced neuronal death. The first type includes two compounds (referred to as 6-1-2 and 6-1-10) that efficiently prevent glutamate or NMDA-induced neuronal death. They also prevent excitotoxicity *in vivo* as assessed by using two animal models of excitotoxicity: acute intoxication with ammonia and a model of cerebral ischemia in rats. Trialkylglycines 6-1-2 and 6-1-10 prevent ammonia-induced (NMDA receptor-mediated) death of mice and neuronal degeneration in the model of cerebral ischemia. The trialkylglycines of the second type act as open channel blockers of the NMDA receptor. The first group of trialkylglycines does not block NMDA receptor channels and does not affect the glutamate-nitric oxide-cGMP pathway. Their molecular target has not yet been identified.

These two types of trialkylglycines (especially those that do not affect NMDA receptor function) might represent effective drugs for the treatment of neurodegeneration. They are likely to be well tolerated and have fewer side effects than NMDA receptor antagonists.

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INTRODUCTION

Excitotoxicity is characterized by an excessive activation of glutamate receptors resulting in neuronal degeneration and death. This overstimulation of receptors can be due to an increased content of glutamate in the synapse, which may occur either because glutamate release is increased or reuptake is diminished. Excitotoxicity may also occur without changes in extracellular glutamate when other factors (e.g., neuronal depolarization) favor excessive activation of NMDA receptors. This seems to be the case in acute ammonia intoxication (14). In some cases exogenous substances may also excessively activate glutamate receptors. One example of these exogenous compounds acting on glutamate receptors is domoic acid, an analog of the excitotoxic amino acids, glutamate and kainic acid, which is produced by diatoms and can accumulate in the food chain. Domoic acid was the neurotoxin present in mussels responsible for a food poisoning incident in Canada that killed several people and left others with memory impairment (3,9).

The molecular mechanisms of glutamate neurotoxicity have not been completely elucidated, but an essential initial event is an increase in intracellular Ca^{2+} in the postsynaptic neurons (6). Massive Ca^{2+} influx leads to an imbalance of calcium homeostasis and to neuronal degeneration and death (25,29). Depending on the kind of receptor involved, degree of receptor activation and neuronal type, excitotoxicity may lead to neuronal death by different mechanisms. In some cases neuronal death is induced by "classical" apoptosis, in others by "classical" necrosis, or by intermediate type of mechanism. Events underlying neuronal cell death induced by overstimulation of glutamate receptors may include: formation of nitric oxide and cGMP (8), production of reactive oxygen species, alterations of mitochondrial potential, decrease of cellular ATP levels and release of apoptosis-inducing factors (22). Excitotoxicity is one of the most extensively studied processes of neuronal death, and plays an important role in many neurodegenerative disorders such as Huntington's (2,32), Parkinson's (30) and Alzheimer's diseases (21,25). Moreover, glutamate toxicity is also involved in neuronal damage in brain ischemia (1,31). NMDA receptors play a major role in mediating excitotoxicity mainly due to their remarkable ability to facilitate calcium entry (6). For this reason, a significant effort has been made to antagonize their action with drugs that target different binding sites on the receptor (4).

NMDA receptors play also an important physiological role in learning and memory formation (24), as well as other important cerebral processes. Therefore, blockade of these receptors can induce cognitive deficits and other important undesirable secondary effects. Drugs such as dizocilpine (MK801) and phencyclidine are channel blockers with very high affinity (IC_{50} in nanomolar range). These drugs are efficient neuroprotectants in neuronal cultures but they are not useful *in vivo* since they display important side effects. Other drugs with lower channel binding affinity such as memantine (IC_{50} in micromolar range) exhibit better therapeutic profile (26). However, memantine has also secondary effects and, when administered chronically, it induces neuronal death (18). There is a large number of compounds that can prevent the neuronal death elicited by an excitotoxic insult in neuronal cultures, but these drugs are ineffective *in vivo*, or are toxic even at therapeutic doses. Hence, it is necessary to develop new therapeutic agents that prevent glutamate neurotoxicity, but with minimal side effects. This might be achieved using molecules that do not act on the NMDA receptor itself but on different cellular targets involved in the neurotoxic process. It is important that compounds used therapeutically do not impair the physiological function of glutamatergic neurotransmission (21).

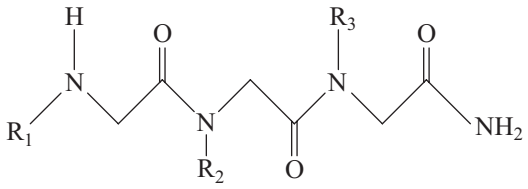

Fig. 1. General structure of trialkylglycines.

Different strategies can be followed to find this kind of compounds and to develop new therapies to prevent or reverse neurotoxic effects. Screening libraries of compounds synthesized by combinatorial chemistry has proven to be a useful way to test thousands of compounds for neuroprotective activity (13,23,27). In this article we review the discovery of neuroprotectant trialkylglycines (peptoids) by two methods of screening. Using one strategy compounds were selected based on their ability to prevent glutamate-induced neuronal death in primary cultures of cerebellar neurons. By the other strategy peptoids were selected on the basis of their ability to block NMDA receptor channels.

CHEMISTRY

Oligomeric N-substituted glycines or "peptoids" are a novel class of polymers that can be used to generate diverse molecular libraries. These compounds are structurally similar to peptides (Fig. 1), but differ from peptides in some respects, such as protease stability. They allow a wide variety of side-chain structures to be incorporated into a molecule with a single linking chemistry facilitating high-throughput synthesis of libraries. These compounds are not only enzymatically but also hydrolytically stable and meet the criteria necessary to be suitable for the generation of libraries (12). Moreover, N-substituted glycines are small molecules (less or equal to 600 Da), with acceptable tissue penetration properties.

The trialkylglycine molecule can be substituted in three positions (R_1, R_2, and R_3) (Fig. 1), with different chemical groups. In our studies 22 amines were used. Trialkylglycine molecule was substituted with each amine in each of the three positions. This strategy produced a total of 66 different mixtures, each of them with an amine in one position and all the combinations of the other amines in the remaining two positions (13). Thus, each mixture contained 484 molecules, giving rise to a chemical diversity of 10648 individual trialkylglycines.

The nature of the amines chosen for the synthesis depends on the end use of the library. There are over 1000 commercially available amines. Generally, if nothing is known about the target protein, a set of amines as diverse as possible should be used. In the case of libraries reviewed here the set of 22 amines included aliphatic and aromatic groups that are likely to improve bioavailability and passage through the blood-brain barrier. The amines used in the synthesis of the library were numbered arbitrarily (from 1 to 22). The compounds synthesized were denoted with the number of the amine in position R_1, R_2 and R_3, respectively (e.g., 6-1-2 indicates amine 6 at position R_1, amine 1 at position R_2; and amine 2 at position R_3).

This review will focus on the characterization of the neuroprotective effects of the two groups of trialkylglycines. Both groups are neuropotective but differ in their mode of

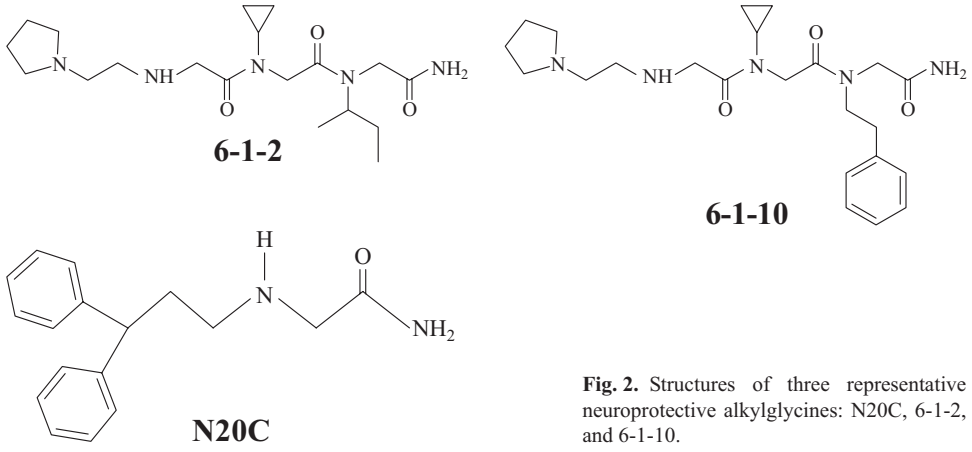

Fig. 2. Structures of three representative neuroprotective alkylglycines: N20C, 6-1-2, and 6-1-10.

action, one group targets and blocks NMDA receptor channel. The other group's target is unknown; the representatives of this second class are the peptoids referred to as 6-1-2 and 6-1-10. Their structures are shown in Fig. 2.

PHARMACOLOGY

Identification of a Group of N-Trialkylglycines with Neuroprotectant Activity

Screening of the N-alkylglycine library to identify neuroprotective peptoids

The library described above was screened to identify neuroprotective peptoids, that prevent glutamate-induced neuronal death. Glutamate neurotoxicity was assayed in cerebellar neurons after 10–15 days in culture. Treatment of cerebellar neurons with 1 mM L-glutamate for 4 h resulted in more than 80% neuronal death. Peptoid mixtures were added to the cultured neurons at 20 min prior to the addition of glutamate. Neuronal viability was determined at 24 h after the treatment by double staining with fluorescein diacetate (labels living cells in green) and propidium iodide (labels dead cells in red). The percentage of surviving neurons was calculated by counting the amount of living and dead neurons under the microscope (10). Using this assay, the first screening of the 66 mixtures identified the amines that afforded better protection when substituted in positions R_1, R_2, and R_3. The mixtures that afforded better neuroprotection were those containing the following fixed amine:

at position R_1, amine 1: cyclopropylamine, or amine 6: 2-N-pyrrolidinylamine;

at position R_2, amine 1: cyclopropylamine, and amine 18: 2-(N-morpholino)ethylamine;

and at position R_3, amine 2: sec-butylamine, or amine 10: 2-phenylethylamine, and amine 17: 2,4-(aminosulphonylphenyl)ethylamine.

Hence, individual compounds were synthesized containing the different combinations of the above-mentioned amines at the indicated positions and their activity in preventing

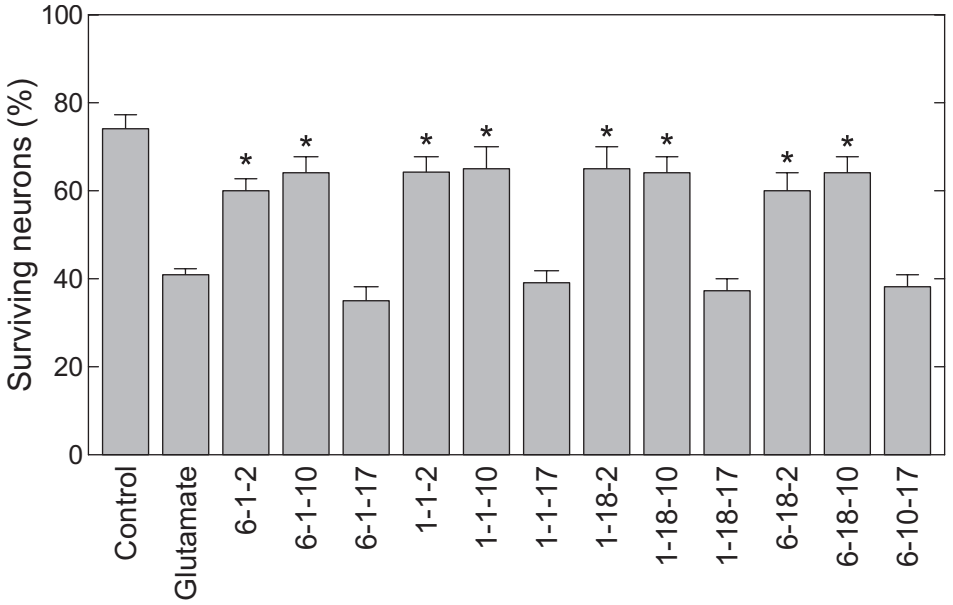

Fig. 3. Prevention by trialkylglycines of glutamate-induced death of cerebellar neurons in primary culture. Cells were used at 10 to 14 days after seeding. *Neuron*s were treated with 1 μM glutamate after preincubation for 15 min with 50 mg/mL of peptoids. Cell viability was determined at 24 h after treatment by staining with fluorescein diacetate and propidium iodide, as previously described (23). The percentage of surviving neurons was calculated by assessing the ratio of fluorescein diacetate/propidium iodide (green/red). At least 1200 cells were counted for each point. Values are given as means ± standard deviations. The values that are significantly different from those in neurons treated with glutamate only are indicated by asterisks ($p < 0.001$). From ref. 23 with permission.

glutamate-induced neuronal death was assayed as above (Fig. 3). In this second screening the twelve individual peptoids were tested at a concentration of 50 mg/mL (approximately 100 μM) and four of them did not show neuroprotective activity when used individually. The peptoids referred to as: 1-1-2; 6-1-2; 1-1-10; 6-1-10; 1-18-2; 6-18-2; 1-18-10; 6-18-10 prevented nearly completely glutamate-induced neuronal death. Dose-dependence of the neuroprotective effect was assessed and it was similar for all effective peptoids. At a concentration of 1 mg/mL they prevented neuronal death by approximately 20%, and the protection was nearly complete at 50 mg/mL (23). The effects of peptoids 6-1-2 and 6-1-10 are shown in Fig. 4.

Since most of the active peptoids exhibited comparable neuroprotective effects two of them 6-1-2 and 6-1-10 (Table 1) were selected to study their neuroprotective effect *in vivo*.

In vivo neuroprotective properties of peptoids 6-1-2 and 6-1-10

Two different animal models of glutamate-mediated neurotoxicity were used to evaluate the neuroprotective effects of the peptoids *in vivo*. One model was acute intoxication with large doses of ammonia. The other was a model of cerebral ischemia, provoked by transient occlusion of the carotids.

Acute ammonium intoxication leads to animal death that is mediated by excessive activation of NMDA receptors (14, 15). Administration of the peptoid 6-1-2 (50 mg/kg)

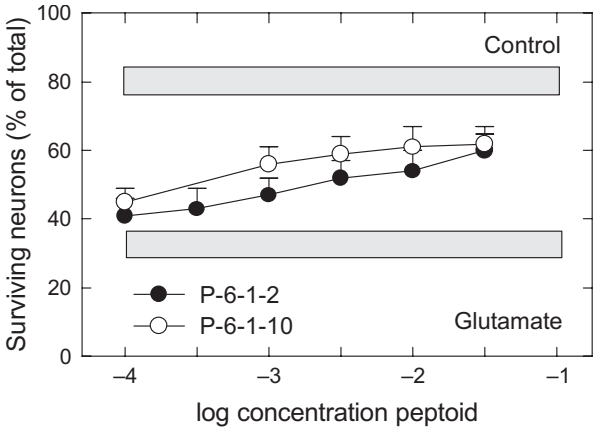

Fig. 4. Concentration dependence of the protective effect of trialkylglycine against glutamate neurotoxicity in primary cultures of neurons. Cells used 10 to 14 days after seeding. Neurons were treated with 1 μM glutamate after preincubation for 15 min with different peptoids at indicated concentrations. Surviving neurons were counted as described in Fig. 3. Data are expressed as means ± standard deviations and are derived from ref. 23.

15 min prior to the injection of ammonium acetate, 14 mmol/kg, protected 100% of the animals, whereas only 16% of mice injected with ammonium acetate alone survived. Peptoid 6-1-10 protected 82% of the mice (Table 1). These results clearly show that peptoids 6-1-2 and 6-1-10 afford a remarkable protection against excitotoxicity in this animal model.

Transient forebrain ischemia was induced using the four-vessel occlusion model of Pulsinelli and Brierly (28), in which the vertebral arteries are cauterized in anesthetized rats at the level of the lateral vertebral foramen. After 24 h, the carotid arteries were clamped for 15 min under halothane anesthesia. To evaluate cerebral damage the animals were sacrificed 7 days later and neuronal degeneration visualized and quantified by the silver impregnation method. One of the brain areas in which this model of ischemia induces massive neurodegeneration is the caudo-putamen (Fig. 5).

Peptoids, administered at 15 min prior to, and at 1 and 24 h after occlusion of carotid arteries prevented ischemia-induced neurodegeneration. Peptoid 6-1-2 produced a 65% decrease in the neurodegeneration in the dorsal caudo-putamen area. Peptoid 6-1-10 afforded even higher protection (82% reduction in neurodegeneration) (Fig. 5). These results demonstrate an important protection by these peptoids, administered intraperitoneally, against ischemia-induced neuronal degeneration.

TABLE 1. Comparison of the neuroprotective effects of trialkylglycines, dizocilpine and memantine

Compound	Prevention of glutamate-induced neuronal death, %	Prevention of ammonium acetate-induced death of mice, %
Dizocilpine	90	75[b]
Memantine	70[a]	40[b]
N20C	65	65
6-1-2	70[c]	82[c]
6-1-10	70[c]	100[c]

a, Values from ref. 21; b, values from ref. 35; c, values from ref. 26.
There was no significant difference among drugs in the degree of protection from glutamate-induced neuronal death. Peptoids and dizocilpine were significantly more effective in protecting mice from the lethal effects of ammonium acetate.

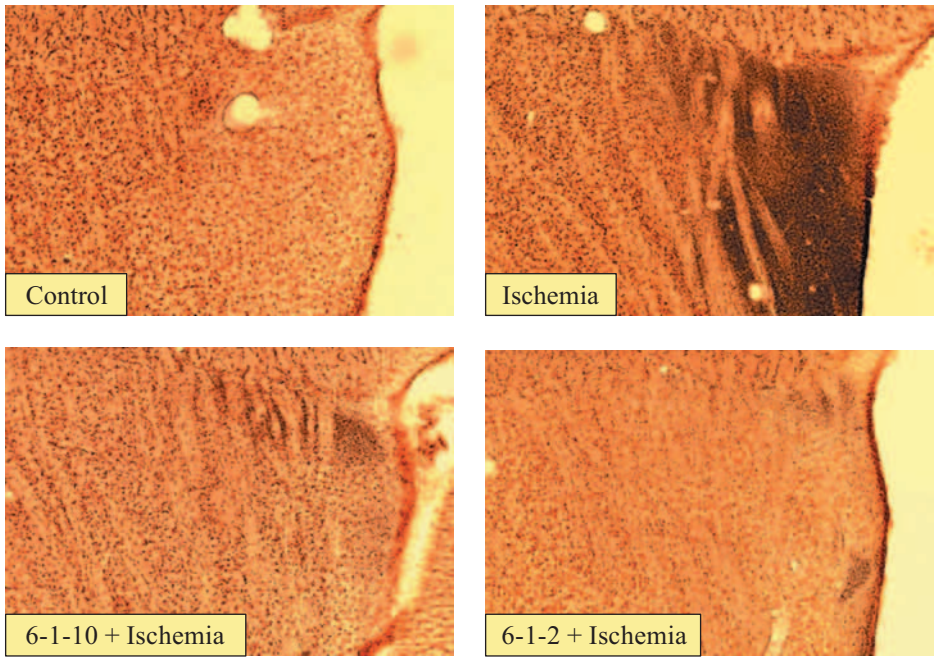

Fig. 5. Peptoids 6-1-2 and 6-1-10 prevent neurodegeneration in striatum induced by ischemia. Rats were subjected to transient ischemia, with or without treatment with peptoids. Cerebral ischemia was induced using the four-vessel occlusion model of Pulsinelli and Brierley (28). Rats received i.p. injection of the peptoids (50 mg/kg) three times: at 15 min before occlusion of the carotid arteries, immediately after removal of the carotid clamp and after 24 h of reperfusion. After 7 days, rats were anesthetized and perfused with 0.9% saline followed by 4% paraformaldehyde in phosphate buffer. Neurodegeneration was visualized using the silver impregnation method. top-left) control rat; top-right) rat subjected to ischemia without treatment; bottom-left) rat treated with peptoid 6-1-10 and subjected to ischemia; bottom-right) rat treated with peptoid 6-1-2 and subjected to ischemia. From ref. 23 with permission.

Studies on the mechanism by which peptoids 6-1-2 and 6-1-10 prevent excitotoxicity

To gain insight into the mechanism by which this group of trialkylglycines produces neuroprotection, we studied their effect on glutamate-induced increase in intracellular Ca^{2+} in cultured cerebellar granule cells. These peptoids were found not to interfere with the increase in intracellular Ca^{2+} induced by NMDA or L-glutamate. It was, therefore, assumed that they do not interfere with the function of NMDA receptors. Also, these compounds did not alter ionic currents induced by activation of recombinant rat brain NMDA receptors expressed in *Xenopus* oocytes. These trialkylglycines have the advantage not to interfere with the physiological activation of NMDA receptors, so that the secondary effects caused by NMDA receptor antagonists can be avoided.

It has been shown that the glutamate-nitric oxide-cGMP signaling pathway is involved in the process by which excessive activation of NMDA receptors leads to neuronal death and that inhibition of any of the steps of this pathway prevents glutamate-induced neurotoxicity (8,20,23). Ca^{2+} entering through NMDA receptors binds to calmodulin and acti-

vates nitric oxide synthase (NOS) thus increasing nitric oxide (NO) formation. NO, in turn, stimulates soluble guanylate cyclase activity, increasing cGMP production.

Inhibition of glutamate-induced formation of nitric oxide or GMP, prevents glutamate-induced neuronal death in cerebellar neurons in culture (16). Thus, it was tested whether the trialkylglycines affect this pathway. The increase in cGMP levels induced by NMDA receptor stimulation in cultured neurons was not affected by preincubation with peptoids 6-1-2 and 6-1-10, confirming that the NMDA receptor channel is not blocked and showing that none of the steps of the glutamate-nitric oxide-cGMP pathway is affected by these peptoids. This suggests that these trialkylglycines act on molecular targets involved in the neurodegeneration process but not on any of the steps of this pathway.

In brains of patients suffering an ischemic episode and in animal models of cerebral ischemia, the damaged areas present a central nucleus of necrotic death and an area surrounding it where cells suffer apoptotic death (7). The signaling events involved in apoptotic death lead to the activation of caspase 3, so that the activated caspase 3 may be used as a marker for apoptotic cells (19). This marker was used to assess whether the trialkylglycines are able to prevent ischemia-induced neuronal apoptosis.

Cells containing activated caspase 3 (apoptotic cells) were visualized and quantified by immunohistochemistry in the striatum of rats 7 days after subjecting them to ischemia with or without treatment with peptoids 6-1-2 or 6-1-10, as described above for the analysis of neurodegeneration by the silver impregnation method. By intraperitoneal injection peptoid 6-1-2 reduced the number of neurons containing activated caspase 3 by 74%. Peptoid 6-1-10 reduced the number of apoptotic neurons by 50%. These results clearly show that these trialkylglycines, injected intraperitoneally, interfere with some step of the process by which cerebral ischemia leads to apoptotic cell death in striatum.

Although further studies are needed to identify the molecular targets of these peptoids, the results showed by Montoliu et al. (23) demonstrate that these trialkylglycines are drugs with a high therapeutic potential for the treatment of excitotoxicity, including that associated with some neurodegenerative disorders. Moreover, they may reach the brain and prevent excitotoxicity when administered systemically. Furthermore, since peptoids 6-1-2 and 6-1-10 do not interfere with NMDA receptor function, their therapeutic utility is clearly better than that of the NMDA channel blockers. They are likely to produce less side effects and to keep physiological glutamatergic neurotransmission unaltered.

Identification of N-Alkylglycines Acting as NMDA Receptor Channel Blockers

Screening of the trialkylglycine library

The second strategy was to screen 66 mixtures in the library of peptoids to assess their ability to block NMDA receptor channels. This was accomplished by measuring the effects of trialkylglycines on glutamate-induced ionic currents through recombinant rat brain NMDA receptor channels expressed in oocytes. Using this approach several peptoid mixtures were identified that blocked at least 50% of glutamate-evoked ionic currents through NMDA receptors. The mixtures more efficient in blocking the currents were those that contained the following amines in the following positions of the trialkylglycine molecule:

at position R_1: 2-(N-methyl)pyrrolidinyl] ethylamine and 3,3-diphenylpropylamine;

at position R_2: 3-(N,N-diethylamino)propylamine and 3,3–diphenylpropylamine;

at position R_3: 2-(methylcarbonylamino)ethyl, 2-(2-pyridyl)ethylamine, 3-(imidazolyl) ethylamine, 3,3-diphenylpropylamine, and 3-(N,N-dimethylamino)propylamine.

Subsequently 8 individual trialkylglycines, containing combinations of the above amines at the indicated positions, were synthesized and assayed for blockade of ionic currents through the NMDA receptor channel. The individual compounds that showed better blocking activity were:

N20-19-7C: [(3,3-diphenylpropyl)glycyl]-[[3-N,N-diethylamino)propyl]glycyl]-[2-(methylcarbonylamino)ethyl] glycinamide;

N20-20-7C: [(3,3-diphenylpropyl)glycyl]-[(3,3-diphenylpropyl)glycyl]-[2-(methylcarbonylamino)ethyl] glycinamide;

N20-19-12C: [(3,3-diphenylpropyl)glycyl]-[[3-(N,N-diethylamino)propyl]glycyl]-[3-(imidazolyl)ethyl] glycinamide.

At 100 μM these molecules blocked ionic currents through NMDA receptor channels by at least 85%. Dose-response studies showed a low binding efficacy. Analysis of current-to-voltage relationships of receptor blockade by these molecules revealed weak voltage dependence, suggesting that these molecules might not reach deep into the channel pore to access their binding site. To circumvent this shortcoming the authors followed a stepwise size reduction strategy to find molecules with improved blocking activity. The 3,3-diphenylpropylamino moiety was preserved, as it seems to be crucial for the blockade of the NMDA receptor channel.

Finally, three molecules were identified, the N-dialkylglycines N20-19C, N20-20C, and the N-alkylglycine N20C. The study of the relationship between structure, activity and chemical stability identified N20C as the best candidate (Fig. 2). since both N-dialkylglycines, although effective neuroprotectants, were chemically unstable giving rise to the corresponding 1,4-diketopiperazines.

Properties of N20C as NMDA receptor channel blockers

N20C (3,3–diphenylpropyl-N-glycinamide) selectively block NMDA receptor channel with an affinity in the micromolar range. Glutamate-evoked ionic currents through NMDA receptors expressed in oocytes were rapidly reduced by more than 80% when 50 μM N20C was added. After removal of this alkylglycine, the recovery of the glutamate-induced response was fast. Dose-response analysis showed that N20C block the currents with an IC_{50} of 5.0 ± 0.2 μM, which is approximately 4 times lower than that of trialkylglycines. The hill-coefficient was 0.8 ± 0.1, suggesting the presence of a single binding site. N20C is highly selective for NMDA receptor, at 50 μM N20C inhibited currents through NMDA receptor channels by 92%. The ionic currents through GluR1 or capsaicin-activated receptor VR_1 were inhibited by N20C by only 5 and 7% respectively (34). The blockade of NMDA receptors by N20C is voltage dependent, inhibiting glutamate/glycine responses only at membrane voltages in the range of –80 to –40 mV. N20C does not act as a competitive antagonist at the glutamate binding site but it seems to bind to the same site as dizocilpine, deep inside the ion channel.

Neuroprotective effects of N20C in cultured neurons

N20C shows significant neuroprotective activity. Treatment of cerebellar granule cells in culture with 1 μM glutamate or NMDA for 4 h results in more that 80% neuronal death. The presence of N20C largely reduced glutamate and NMDA-induced neuronal death.

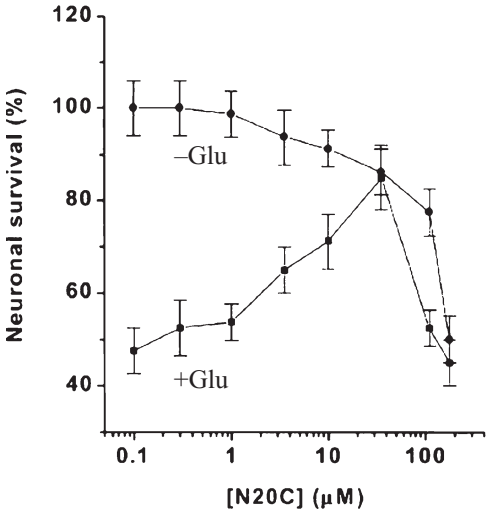

Fig. 6. N20C prevents glutamate-induced neuronal death. Primary cultures of cerebellar neurons were treated with N20C at indicated concentrations. Some samples (indicated as "+Glu") were treated with glutamate 1 μM, added 20 min after N20C. Other samples (indicated as "–Glu") were treated only with N20C. Cell viability was determined 24 h after treatment by staining with fluorescein diacetate and propidium iodide as previously described (23). The percentage of surviving neurons was calculated by assessing the ratio of fluorescein diacetate/propidium iodide (green/red). At least 1200 cells were counted for each point. Values are given as means ± S.E.M. It can be seen that N20C prevents glutamate neurotoxicity, but is toxic at high concentrations. From ref. 27.

This neuroprotective effect increased with the drug concentration that ranged from 0.1 to 30 μM. At higher concentrations this compound was toxic to the cells, causing by itself high incidence of neuronal death (Fig. 6). Some trialkylglycines, identified using this screening procedure, were also neuroprotective but the concentrations required to achieve efficient protective effects were much higher (100 μM).

To confirm that N20C blocks the NMDA receptor channel also in neurons we attempted to prevent with N20C the increase in intracellular Ca^{2+} induced by activation of NMDA receptors in cerebellar neurons in culture. Incubation with N20C effectively inhibited NMDA-induced Ca^{2+} increase in the neurons and reduced in a concentration-dependent manner NMDA-induced formation of cGMP. Moreover, there was a good correlation between the prevention of neuronal death and the reduction in glutamate-induced Ca^{2+} rise produced by this alkylglycine at different concentrations. These results suggested that N20C prevents neuronal death by interfering with NMDA receptor activation and entry of Ca^{2+} through its channel into the postsynaptic neurons.

Neuroprotective effects of N20C in vivo

To assess whether the alkylglycine N20C exhibited *in vivo* neuroprotection, an animal model of severe excitotoxicity caused by acute injection of ammonium acetate 7 mmol/kg was used. By intraperitoneal injection large doses of ammonium acetate cause animal death. The death is likely to be due to excessive stimulation of NMDA receptors (14), and is completely prevented by different NMDA receptor antagonists (15). N20C administered i.p. at 10 min before ammonium acetate injection prevented animal death in a dose-dependent manner. The minimal protective dose was 5 mg/kg and at 50 mg/kg it protected 70% of mice injected with ammonium acetate (Fig. 7). This finding indicates that N20C can cross blood-brain barrier and act on the NMDA receptors in the brain. The neuroprotection afforded by N20C is significantly higher than that afforded by memantine under similar conditions (Table 1).

It is interesting to point out that at concentrations higher than 100 mg/g N20C did not exert toxic effects, and that animals treated with this N-alkylglycine did not display motor

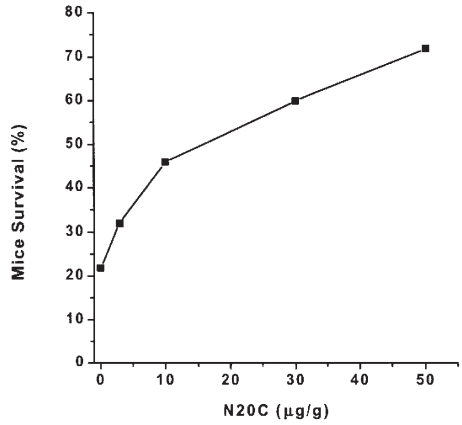

Fig. 7. N20C prevents ammoniammonium acetate-induced death of mice. The protective effect of N20C *in vivo* was assessed using an animal model of excitotoxicity in which mice are injecting i.p. with 14 mmol/kg of ammonium acetate. This leads to death of most mice by a mechanism mediated by excessive activation of NMDA receptors. N20C was injected i.p. 10 min prior to ammonium acetate. The number of surviving mice was counted at 24 h after injection of ammonium acetate. 38 mice were used in the control group. Nine to 25 mice were used at each of the treatment groups. Protection was significant at all doses of N20C used. From ref. 27.

insufficiency or noticeable changes in behavior. Overall, the *in vivo* neuroprotective effects of N20C were significantly better than those of N20-19-7C and N20-20-7C which required doses of 200 mg/kg to protect animals against ammonium acetate toxicity (27).

All the above reported studies involved pretreatment with the drugs prior to challenging neurons with glutamate, or animals treated with ammonium acetate or with induced cerebral ischemia. It has, therefore, not been established whether the drugs can reverse neurodegeneration. Studies to clarify this point are in progress. In any case, it is clear, from the studies on animals subjected to cerebral ischemia, that peptoids prevent the progression of neurodegeneration, suggesting that they can have therapeutic utility in this situation. The possible side effects of the trialkylglycines remain to be determined, but it is evident that peptoids such as 6-1-2 or 6-1-10, that do not block NMDA receptor function, are not likely to produce side effects of typical NMDA receptor antagonists in clinical trials. Whether the trialkylglycines will be well tolerated is currently under study.

In summary, the trialkylglycines reviewed here, represent a new class of neuroprotective molecules that may lead to the development of new therapeutic agents for the treatment of disorders involving excessive activation of glutamate receptors, including. neuronal damage following cerebral ischemia and several neurodegenerative and neuropsychiatric diseases. The trialkylglycines that do not interfere with the physiological function of NMDA receptors seem particularly promising since they prevent necrotic as well as apoptotic neuronal death and since they are expected to produce less side effects than other neuroprotective agents.

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