REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978

PUBLICATION PARTICULARS AND ABSTRACT

(Section 32(3)(a) - Regulations 22(i)(g) and 31)

OFFICIAL APPLICATION NO.		LODGING DATE		ACCEPTANCE DATE	
21	01 2002/0539	23	22 January 2002	43	22.7.2002

INTERNATIONAL CLASSIFICATION	
51	A61K

NOT FOR PUBLICATION
CLASSIFIED BY :

FULL NAME(S) OF APPLICANT(S)	
71	~~IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE~~ AANSOEKER SE VERVANG APPLICANTS SUBSTITUTED PROTEXEON LIMITED 4/6/02

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EARLIEST PRIORITY CLAIMED	COUNTRY		NUMBER		DATE	
NOTE : The country must be indicated by its International Abbreviation - see Schedule 4 of the Regulations.	33	GB	31	9917822.0	32	29 July 1999

TITLE OF INVENTION	
54	XENON AND NMDA ANTAGONIST

57	ABSTRACT (NOT MORE THAN 150 WORDS)	NUMBER OF PAGES	60

FOR ABSTRACT SEE THE NEXT SHEET

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(57) **Abstract:** The present invention relates to the use of xenon as a neuroprotectant and/or as an inhibitor of synaptic plasticity. In a preferred aspect, the xenon acts as an NMDA antagonist. The present invention also provides a method of reducing the level of activation of the NMDA receptors in a mammal, the method comprising modulating the activity of the NMDA receptor by administering to the mammal a therapeutically effective amount of xenon, wherein said reduction achieves neuroprotection and/or an inhibition of synaptic plasticity. A further embodiment of the invention provides a pharmaceutical composition for providing neuroprotection and/or inhibition of synaptic plasticity, together with a process for the process for the preparation thereof.

XENON AN NMDA ANTAGONIST

Background

The present invention relates to the use of xenon as a neuroprotectant and/or in the inhibition of synaptic plasticity. In particular, the invention relates to the use of xenon as an NMDA antagonist, and its use in the treatment of conditions associated with NMDA receptor activity.

The NMDA (N-methyl-D-aspartate) receptor is a major subclass of glutamate receptor and glutamate is believed to be the most important excitatory neurotransmitter in the mammalian central nervous system. Importantly, activation of the NMDA receptor has been shown to be the central event which leads to excitotoxicity and neuronal death in many disease states, as well as a result of hypoxia and ischaemia following head trauma, stroke and following cardiac arrest.

It is known in the art that the NMDA receptor plays a major role in the synaptic plasticity which underlies many higher cognitive functions, such as memory and learning, as well as in certain nociceptive pathways and in the perception of pain (Collingridge *et al*, The NMDA Receptor, Oxford University Press, 1994). In addition, certain properties of NMDA receptors suggest that they may be involved in the information-processing in the brain which underlies consciousness itself.

NMDA receptor antagonists are therapeutically valuable for a number of reasons, such as the following three specific reasons. Firstly, NMDA receptor antagonists confer profound analgesia, a highly desirable component of general anaesthesia and sedation. Secondly, NMDA receptor antagonists are neuroprotective under many clinically relevant circumstances (including ischemia, brain trauma, neuropathic pain states, and certain types of convulsions). Thirdly, NMDA receptor antagonists confer a valuable degree of amnesia.

However, it is clear from the prior art that there are a number of drawbacks associated with current NMDA receptor antagonists. These include the production of involuntary movements, stimulation of the sympathetic nervous system, induction of neurotoxicity at high doses (which is pertinent since NMDA receptor antagonists have low potencies as general anaesthetics), depression of the myocardium, and proconvulsions in some epileptogenic paradigms e.g., "kindling" (Wlaz P *et al*, Eur. J. Neurosci. 1994; 6:1710-1719). In particular, there have been considerable difficulties in developing new NMDA receptor antagonists that are able to cross the blood-brain barrier. This factor has also limited the therapeutic applications of many known NMDA antagonists.

The present invention thus seeks to provide an improved NMDA receptor antagonist for general pharmaceutical use which can readily diffuse across the blood brain barrier.

Statement of Invention

Aspects of the present invention are presented in the accompanying claims and in the following description.

Thus, the present invention relates to the use of xenon as a neuroprotectant and/or as an inhibitor of synaptic plasticity. In a preferred aspect, the xenon acts as an NMDA receptor antagonist.

A second aspect of the present invention relates to a method of treatment comprising modulating the activity of an NMDA receptor in a mammal, the method comprising modulating the activity of the NMDA receptor by administering to the mammal a therapeutically effective amount of xenon.

In a preferred aspect of the invention, the xenon is administered in combination with a pharmaceutically acceptable carrier, diluent or excipient.

Preferably, the method of treatment may be used to treat a mammal suffering from a condition associated with NMDA receptor activity. In a more preferred aspect, the mammal is treated for a condition that is associated with NMDA receptor activation.

Even more preferably, the present invention relates to a method of treatment wherein the xenon reduces the level of activation of the NMDA receptor.

Another embodiment of the invention relates to a process for the preparation of a pharmaceutical composition suitable for modulating the activity of an NMDA receptor, which process comprises adding an NMDA antagonist to a pharmaceutically acceptable carrier, excipient or diluent, wherein the improvement comprises using xenon as the NMDA antagonist.

A further embodiment of the present invention provides a pharmaceutical composition for modulating NMDA activity which comprises an NMDA antagonist and a pharmaceutically acceptable carrier, excipient or diluent, wherein the improvement comprises using xenon as the NMDA antagonist.

Yet another embodiment of the invention relates to the use of xenon in the preparation of a pharmaceutical for use in modulating the activity of an NMDA receptor in a mammal.

In a preferred aspect, the xenon in the pharmaceutical is used in combination with a pharmaceutically acceptable carrier, diluent or excipient.

Preferably, the pharmaceutical is for use in treating a condition associated with NMDA receptor activity. In a more preferred aspect, the pharmaceutical is for use in treating a condition associated with NMDA receptor activation. Even more preferably, the pharmaceutical is for use in reducing the level of activation of the NMDA receptor.

Detailed Description

In a broad aspect, the present invention relates to the use of xenon as a neuroprotectant and/or as an inhibitor of synaptic plasticity. In a preferred aspect, the xenon acts as an NMDA receptor antagonist.

Xenon is a chemically inert gas whose anaesthetic properties have been known for over 50 years (Lawrence JH *et al*, J. Physiol. 1946; 105:197-204). Since its first use in surgery (Cullen SC *et al*, Science 1951; 113:580-582), a number of research groups have shown it has an excellent pharmacological profile, including the absence of metabolic by-products, profound analgesia, rapid onset and recovery, and minimal effects on the cardiovascular system (Lachmann B *et al*, Lancet 1990; 335:1413-1415; Kennedy RR *et al*, Anaesth. Intens. Care 1992; 20:66-70; Luttropp HH *et al*, Acta Anaesthesiol. Scand. 1994; 38:121-125; Goto T *et al*, Anesthesiology 1997; 86:1273-1278; Marx T *et al*, Br. J. Anaesth. 1997; 78:326-327). However, up to now, the molecular mechanisms underlying the clinical activity of xenon have remained elusive.

We have described the use of xenon in a pharmaceutical application in United Kingdom patent application number GB 9913677.2 (filed 11 June 1999), the contents of which are incorporated herein by reference.

The term "anatagonist" is used in its normal sense in the art, i.e., a chemical compound which prevents functional activation of a receptor by its natural agonist (glutamate, in this case).

It is widely known that most other anaesthetics enhance the activity of inhibitory GABA (γ-aminobutyric acid type-A) receptors (Franks NP *et al* Nature 1994; 367: 607-614; Mihic SJ *et al*, Nature 1997; 389: 385-389). However, the effect of xenon on these receptors is believed to be negligible. Instead, xenon potently inhibits the excitatory NMDA receptor channels. This effect accounts for many of the important

features of its pharmacological profile and is likely to be instrumental in the anaesthetic and analgesic effects of this inert gas.

It is to be noted that the prior art has neither disclosed nor suggested the use of xenon as a neuroprotectant and/or as an inhibitor of synaptic plasticity. Nor has the prior art disclosed or suggested the use of xenon as an NMDA receptor antagonist.

Unlike many other NMDA antagonists, xenon is able to rapidly equilibrate with the brain by diffusing across the blood brain barrier. A further advantage of using xenon as an NMDA antagonist is that the molecule is an inert, volatile gas that can be rapidly eliminated via respiration.

Thus, the present invention relates to the use of xenon to achieve one or more of the following:

a) to provide neuroprotection;

b) to inhibit synaptic plasticity, e.g. to inhibit the development of tolerance to opiates.

The term "neuroprotection" means protecting a neural entity, such as a neuron, at a site of injury, for example, an ischaemic injury or traumatic injury.

In a further embodiment, the invention provides a method of providing neuroprotection and/or inhibiting synaptic plasticity in a mammal, the method comprising administering to the mammal a therapeutically effective amount of xenon.

Preferably, the xenon is administered in combination with a pharmaceutically acceptable carrier, diluent or excipient. By way of example, in the pharmaceutical compositions of the present invention, xenon may be admixed with any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s)

selected with regard to the intended route of administration and standard pharmaceutical practice.

The xenon may also be administered in combination with another pharmaceutically active agent. The agent may be any suitable pharmaceutically active agent including anaesthetic or sedative agents which promote GABAergic activity. Examples of such GABAergic agents include isoflurane, propofol and benzodiazapines.

In another embodiment, the invention provides a method of reducing the level of activation of the NMDA receptors in a mammal, the method comprising modulating the activity of the NMDA receptor by administering to the mammal a therapeutically effective amount of xenon, wherein said reduction achieves neuroprotection and/or an inhibition of synaptic plasticity.

A further embodiment of the invention provides a process for the preparation of a pharmaceutical composition suitable for neuroprotection and/or inhibition of synaptic plasticity, which process comprises adding xenon to a pharmaceutically acceptable carrier, excipient or diluent, wherein the improvement comprises using xenon as a neuroprotectant and/or an inhibitor of synaptic plasticity.

In another embodiment, the invention provides a pharmaceutical composition for neuroprotection and/or an inhibition of synaptic plasticity which comprises xenon and a pharmaceutically acceptable carrier, excipient or diluent, wherein the improvement comprises using xenon as a neuroprotectant and/or an inhibitor of synaptic plasticity.

Typically, the pharmaceutical compositions of the present invention may be delivered intravenously (either by bolus administration or infusion), neuraxially (either subdural or subarachnoid), transdermally, or by inhalation.

By way of example, the present invention also relates to the use of xenon in the preparation of a medicament for any one or more of the following:

a) to provide neuroprotection;

b) to inhibit synaptic plasticity, e.g. to inhibit the development of tolerance to opiates.

The concentration of xenon employed in the composition may be the minimum concentration required to achieve the desired clinical effect. It is usual for a physician to determine the actual dosage that will be most suitable for an individual patient, and this dose will vary with the age, weight and response of the particular patient. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

The pharmaceutical composition of the present invention may be for human administration or animal administration.

Thus, the composition of the present invention may also be used as an animal medicament. In this regard, the invention further relates to a veterinary composition comprising the composition of the present invention and a veterinarily acceptable diluent, excipient or carrier.

For veterinary use, the composition of the present invention, or a veterinarily acceptable composition thereof, is typically administered in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular animal.

The invention is further described with reference to the accompanying figures wherein:

Figure 1 shows control excitatory and inhibitory postsynaptic responses.

Figure 2 shows representative traces illustrating the effects of xenon on inhibitory and excitatory postsynaptic responses.

Figure 3 shows the percentage of maximum control current against the concentration of NMDA in the presence and absence of xenon.

Figure 4 shows the effect of AP5 and xenon on excitatory postsynaptic response, and inset, the NMDA-receptor-mediated component.

Figure 5 shows the construction and performance of the apparatus used for exposing cells in culture.

Figure 6 shows an overview of the entire chamber used for exposing cells in culture.

Figure 7 shows the chamber kinetics for a typical experiment in which xenon is introduced into the chamber, showing the xenon increase and nitrogen decrease against time (in minutes).

Figure 8 shows a calibration chart for xenon.

Figure 9 shows a typical example of a gas analysis from the chamber.

Figure 10 shows a dose response curve of NMDA-induced neuronal injury.

Figure 11 shows a dose response curve of glutamate-induced neuronal injury.

Figure 12 shows the effect of 75% xenon during and after NMDA-induced neuronal injury.

Figure 13 shows the effect of 75% xenon during and after glutamate-induced neuronal injury.

Figure 14 shows the effect of increasing doses of Prostaglandin E_1 (PGE_1) on adenylyl cyclase (AC).

Figure 15 shows the effect of coadministration of norepinephrine on adenylyl cyclase (AC) activity.

In more detail, Figure 1 shows A) A representative excitatory postsynaptic current (EPSC) and its sensitivity to 1 mM kynurenic acid and insensitivity to 10 μM bicuculline. The inset shows the linear current-voltage relationship for the peak of the EPSC, normalized to the current measured at -60 mV; the data points represent mean values (for an average of 5 cells). B) A representative inhibitory postsynaptic current (IPSC) and its sensitivity to 10 μM bicuculline and insensitivity to 1 mM kynurenic acid. The inset shows the linear current-voltage relationship for the peak of the IPSC, normalized to the current measured at -60 mV; the data points represent mean values (for an average of 6 cells). In the insets the errors bars are SEMs, but these are not shown where they are smaller than the size of the symbol.

In more detail, Figure 2 shows A) GABAergic postsynaptic currents in the presence and absence of 3.4 mM xenon. Xenon has no significant effect on the IPSC. The inset shows representative traces illustrating the lack of an effect of 4.3 mM xenon on the current evoked by 3 μM GABA. B) Glutamatergic postsynaptic currents in the presence and absence of 3.4 mM xenon. The principal effect is a reduction in the slow component of the current (in this example by about 70%). The dots represent biexponential fits to the measured currents. The inset shows representative traces illustrating the effect of 3.4 mM xenon on the current evoked by 100 μM NMDA.

In more detail, Figure 3 shows that NMDA activates an inward current (in neurons clamped at -60 mV) with an EC_{50} of 24 ± 2 μM NMDA and a Hill coefficient of 1.2 ± 0.1. Xenon inhibits the current by approximately 60 % but does not significantly

change either the EC_{50} or the Hill coefficient. Each data point represents the mean peak current from at least 6 cells.

In more detail, Figure 4 shows that xenon selectively inhibits the NMDA-receptor-mediated component of glutamatergic excitatory postsynaptic currents (EPCSs). Neurons were voltage-clamped at -60 mV; synaptic responses were stimulated by a 2-ms depolarising pulse to +20 mV. Control glutamatergic EPSCs displayed a characteristic biphasic decay. The slow component was completely blocked by 200 μM AP5, leaving the fast component almost unaffected. Inset, the NMDA-receptor-mediated component (the difference between the control EPCS and that in the presence of AP5) and its size in the presence of xenon (calculated by taking the difference between the EPSC in the presence of xenon and that in the presence of AP5). Control solutions were equilibrated at room temperature with 80 % N_2 and 20 % O_2, and the test solutions with 80 % xenon and 20 % O_2.

In more detail figure 5 shows details of the faceplate and fan assembly, gas and electrical feedthroughs. The chamber is fabricated from stainless steel or anodised aluminium and can be easily sterilised. The gases enter through a port coupled to a high-speed fan that provides efficient mixing to ensure the cells are exposed to precisely defined concentrations of xenon. If xenon is introduced into the chamber at v ml/min and the chamber has a volume of V and the concentration of the xenon entering the chamber is c_{in} then the concentration of the gas coming out c_{out} is given by:

$$c_{out} = c_{in}\left[1 - e^{-\frac{v}{V}t}\right]$$

In more detail, Figure 7 shows an experimental test illustrating that the xenon replaces the nitrogen at exactly the theoretical rate. In the presence of one culture plate of cells the volume of gas is 570 mls. So, for a rate of 40 ml xenon/min and a

single culture plate of cells, the gas in the chamber should theoretically reach 95% of the final concentration when t = 43 minutes if the gases are perfectly mixed.

In more detail, Figure 8 is an example of a calibration chart for xenon, showing height of ball in flowmeter (mm) against the xenon flow rate (mls/minute). The composition of the gaseous environment to which the cells were exposed was defined using precision flow rotameters that had been calibrated for xenon (or alternatively for nitrogen, oxygen or carbon dioxide).

In more detail, Figure 9 shows a gas analysis when a defined mixture of gases (xenon, oxygen, nitrogen and carbon dioxide) was passed through a chamber that mixed the gases, providing a stable and precisely controlled environment. The composition of the gases was confirmed using gas chromatography. The gases were sampled at the outlet and sealed in a glass vial. The vial was transferred to a Perkin Elmer automatic headspace sampler HS 40XL which sampled the gases in the vial (40 µl sampling volume) and these were then fed to a Perkin Elmer XL gas chromatograph fitted with a 2 metre x 1/8ss Chromosorb 102 80-100 Mesh column run at 60 °C with helium as the carrier gas (2 mls/min). The gases were detected using a thermal conductivity detector at 110 °C with an injector temperature of 150°C.

In more detail, Figure 10 shows a dose response curve of NMDA-induced neuronal injury in a 15 day old co-culture. Control experiments demonstrated that the underlying glia-monolayer is not affected by NMDA at the maximum dose used (Treatment: 10 minutes, LDH-release after 24 hours).

In more detail, Figure 11 shows a dose response curve of glutamate-induced neuronal injury in a 15 day old co-culture. Control experiments demonstrated that the underlying glia-monolayer is not affected by glutamate at the maximum dose used (Treatment: 30 minutes, LDH-release after 6 hours).

In more detail, Figure 12 shows the effect of 75 % xenon during and after NMDA-induced neuronal injury in which sister cultures (n = 3) were exposed to NMDA (750μmol for 10 minutes) and subsequent neuronal damage was quantified by the appearance of LDH 24 hours after exposure.

In more detail, Figure 13 shows the effect of 75 % xenon during and after glutamate-induced neuronal injury in which sister cultures (n = 3) were exposed to glutamate (75 μmol for 10 minutes) and subsequent neuronal damage was quantified by the appearance of LDH 24 hours after exposure.

In more detail, Figure 14 shows that increasing doses of PGE_1 dose-dependently stimulates adenylyl cyclase (AC).

In more detail, Figure 15 shows that when the alpha-2 agonist norepinephrine is coadministered under conditions in which the AC activity is stimulated with PGE_1, AC activity is dose-dependently inhibited by norepinephrine. Even under basal conditions ("-PGE_1") the inhibitory effect is evident.

Studies carried out by the applicant have shown that xenon has virtually no effect on $GABA_A$ receptors. Currents activated by 3 μM GABA, both in voltage-clamped cultured rat hippocampal neurons and in voltage-clamped PA3 cells (Hadingham KL *et al*, Proc. Natl. Acad. Sci. USA, 1992; 89, 6378-6382) that stably expressed defined GABA subunits, were not significantly affected, even by 100 % xenon. The significance of this observation becomes apparent, given that in order to function as a human anaesthetic, the half maximal effective concentration EC_{50} is 71 % v/v (Cullen *et al*, Anesthsiology 1969, 305-309). Xenon was also shown to have little effect on functional GABA-releasing synapses in hippocampal neurons, with 80 % xenon reducing peak inhibitory post-synaptic currents by only 8 ± 2 %, indicating that the presynaptic effects of xenon must be very modest.

In contrast, xenon was shown to have a significant effect on NMDA receptors. Mechanistic studies on cultured hippocampal neurons have shown that 80% xenon, (which will maintain surgical anaesthesia) reduces NMDA-activated currents by up to 60%, with no significant change in the NMDA EC_{50} value or Hill coefficient. This non-competitive inhibition indicates that xenon should strongly inhibit neural transmission, despite the high glutamate concentrations in synaptic clefts.

Further studies were carried out using microisland cultures of hippocampal neurons that form synapses with themselves (autapses) (Bekkers JM *et al*, Proc. Natl. Acad. Sci. USA, 1991; 88, 7834-7838). A typical glutamatergic postsynaptic current recorded from a hippocampal neuron is shown in Figure 4. The control records show a characteristic biphasic time course, with a fast component mediated by non-NMDA receptors and a much slower component mediated by NMDA receptors. The NMDA receptor mediated component is readily identified as it is blocked by the highly selective competitive antagonist AP5, DL-2-amino-5-phosphononpentanoate (Watkins JC *et al*, Annu. Rev. Pharmacol. Toxicol. 1981; 21, 165-204).

Addition of 200 μM AP5 was shown to almost completely block the slow component, leaving only a fast component, with a single exponential time course very similar to that of the control fast component. The effect of xenon on the glutamatergic postsynaptic current resembles that of AP5 (Figure 4). . The slow NMDA-receptor-mediated component is reduced by over 70 %, whereas the fast component barely changes. So, not only does xenon inhibit synaptic NMDA receptors, it has little apparent effect on non-NMDA receptors.

In terms of the effect on synaptic currents, the selectivity of action observed with xenon is unexpected. In view of the fact that almost all general anaesthetics potentiate the actions of GABA at $GABA_A$ receptors (Tanelian DL *et al*, Anesthesiology 1993; 78: 757-776; Franks NP *et al*, Nature 1994; 367:607-614), it was anticipated that xenon would be no exception. However, the complete absence of an effect of xenon on $GABA_A$ receptors puts it into the same class of agents as ketamine, a so-called

"dissociative" anaesthetic, which is also ineffective at $GABA_A$ receptors (Brockmeyer DM *et al*, Br. J. Anesesth. 1995; 74:79-84) and is thought to act predominantly at NMDA receptors. Similarly, results have shown that xenon selectively blocks NMDA receptors with little effect at AMPA/KA receptors. This latter result strongly suggests that the actions of xenon are postsynaptic in origin. The lack of an effect of xenon on the decay time of the NMDA receptor component, however, rules out a simple open channel block mechanism of inhibition, as does the observation that the EC_{50} concentration for NMDA-evoked currents does not change in the presence of xenon. Whatever the exact molecular basis for the surprising selectivity of xenon for NMDA receptors, it rather simply accounts for many features of its unusual pharmacological profile, including the ability to induce profound analgesia and psychotomimetic effects.

It is thus clear that xenon is surprisingly selective in its action, having very different effects on excitatory and inhibitory synaptic transmission. The action of xenon may be accounted for solely in terms of effects at glutamatergic synapses, although other targets may well be identified in the future. Nonetheless, the insensitivity of GABAergic synapses to xenon indicates that its mechanisms of action are clearly different to those of most general anaesthetics. At the mechanistic level, it is clear that for xenon, postsynaptic receptors are the most important molecular targets.

The present invention is further described by way of example.

Examples

Culturing hippocampal neurons

Hippocampal neurons were grown in culture using methods described previously (Segal MM *et al*, J. Neurophysiol. 1990, 64:1390-1399; Bekkers JM *et al*, Proc. Natl. Acad. Sci. U.S.A. 1991, 88:7834-7838; Mennerick S *et al*, J. Neurophysiol. 1995, 73:320-332). Briefly, hippocampi from Sprague Dawley rats (postnatal day 1-3) were

dissected, roughly sliced and agitated in a papain-containing solution (20 units ml^{-1}) for 30 minutes at 37EC. After washing with enzyme-free solution, the tissue was gently triturated with a fire-polished Pasteur pipette and the cells were plated out at a density of 8 - 10 x 10^4 cells ml^{-1} and cultured (95% air/5% CO_2) at 37°C. Glass coverslips used for culturing the cells were first coated with agarose (0.15% w/v) and then sprayed with a fine mist of poly-D-lysine (PDL) and collagen (0.1 mg ml^{-1} PDL and 0.5 mg ml^{-1} rat-tail collagen) from a glass micro-atomiser and sterilized by UV exposure. This produced micro-islands of permissive substrate with diameters of between 100 and 1000 μm. At 3 - 4 days after plating, when the glial cell layer was about 80% confluent, an anti-mitotic agent (cytosine β-D-arabinofuranoside, 5 μM) was added to arrest glial cell proliferation. Neuronal cultures were then allowed to mature for another 4 - 9 days. We used micro-islands which contained single isolated neurons whose axonal processes and dendritic trees formed multiple self-synapses (autapses). This procedure provided a large population of phenotypically identical monosynaptic connections.

Electrophysiology

The neurons were voltage-clamped using the whole-cell recording technique (Axopatch 200 amplifier, Axon Instruments, Foster City, CA). Electrodes were fabricated from borosilicate glass and typically had resistances of between 3 - 5 MΩ. Series resistance was compensated by 75 - 90%. Neurons were voltage-clamped at -60 mV, and synaptic responses were stimulated by a 2 ms depolarising pulse to +20 mV. Shortly after the restoration of the membrane potential to -60 mV, a large (1 - 20 nA) postsynaptic current was observed and recorded. For the synaptic measurements, data were sampled at 50 kHz, filtered at 20 kHz (-3 dB, 8-pole Bessel) and stored on a computer. The extracellular recording solution was (mM) 137 NaCl, 5 KCl, 3 $CaCl_2$, 5 HEPES, 10 glucose, 0.001 glycine, 0.0001 strychnine-HCl, titrated to pH 7.3 with

NaOH; and the intracellular (pipette) solution was (mM) 140 KCl, 4 NaCl, 0.5 EGTA, 2 MgATP, 10 HEPES, titrated to pH 7.25 with KOH.

For the experiments where GABA, NMDA or glutamate were exogenously applied, the neurons were grown in mass culture and used 3 - 11 days after plating. Data were sampled at 200 Hz and filtered at 100 Hz (-3 dB, 8-pole Bessel). The extracellular recording solution for NMDA- and glutamate-evoked responses was (mM) 150 NaCl, 4 KCl, 2 $CaCl_2$, 10 HEPES, 10 glucose, 0.0002 tetrodotoxin citrate (Tocris Cookson, Bristol, UK), 0.1 picrotoxin, 0.0001 strychnine-HCl, 0.001 glycine, titrated to pH 7.40 with NaOH; the extracellular recording solution for GABA-evoked responses was (mM) 150 NaCl, 4 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 glucose, 0.0002 tetrodotoxin citrate, titrated to pH 7.40 with NaOH; and the intracellular (pipette) solution for GABA-, NMDA- and glutamate-evoked responses was (mM) 140 CsCl, 3 NaCl, 11 EGTA, 2 MgATP, 10 HEPES, titrated to pH 7.20 with CsOH. Unless otherwise stated, all chemicals were obtained from Sigma Chemical Co. (Poole, Dorset, UK). Test solutions were applied to the cells using a rapid-switching perfusion system (Downie DL *et al*, Br. J. Pharmacol. 1996, 118:493-502). All electrophysiological measurements were carried out at room temperature (20 - 23°C).

Preparation of anaesthetic solutions

Xenon solutions were prepared by first bubbling pure gases (oxygen, nitrogen or xenon) through fine sintered-glass bubblers in 250- or 500-ml Drechsel bottles filled with extracellular recording saline. Solutions were bubbled for 1.5 - 2 hours, although equilibrium was found to occur within 45 minutes. (To minimise oxidization, the neurotoxins and neurotransmitters were excluded from the fully oxygenated saline.) During bubbling, the solutions were continually stirred at room temperature. These solutions were then mixed to achieve the desired final concentrations of the gases. Our control solutions usually contained 80% of the nitrogen solution and 20% of the oxygen solution, while our test solutions usually contained 80% of the xenon solution and 20% of the oxygen solution. Using a Bunsen water/gas partition coefficient of

0.0965 (Smith RA *et al*, Biochim. Biophys. Acta 1981, 645:327-338) we calculate that our standard test solution contained 3.4 mM xenon. Xenon (research grade, 99.993% pure) was supplied by BOC gases, Guildford, Surrey, UK. In all cases xenon was pre-applied to the neurons for at least 30 seconds before the initiation of synaptic currents.

Integration of the synaptic responses

In order to obtain an estimate for the total charge transfer, the EPSCs or IPSCs were numerically integrated. However, because in some cases the currents had not decayed to baseline by the end of the recording period, a correction (which was invariably less than 5% of the total charge transfer) was applied by extrapolating the observed current to the baseline using a biexponential fit to the decay phase of the response.

Control synaptic currents

The control synaptic currents were characterised and almost invariably fell into one of two populations, in accordance with the findings of previous studies (Bekkers JM *et al*, Proc. Natl. Acad. Sci. U.S.A. 1991, 88:7834-7838; Mennerick S *et al*, J. Neurophysiol. 1995, 73:320-332). Approximately half of the cells exhibited postsynaptic currents which decayed relatively rapidly (~8 ms half-time), while the other half exhibited currents that were markedly slower (~40 ms half-time) (see Fig. 1 and Table 1). The more rapid responses were recorded from cells with a rounded appearance and complex dendritic trees. Their sensitivity to kynurenic acid (80 ± 3% inhibition of the peak current with 1 mM kynurenic acid; n = 7 cells) and insensitivity to bicuculline (0.4 ± 1.3% inhibition of the peak current with 10 μM bicuculline; n = 13 cells) identifies these cells as excitatory glutamatergic neurons. In contrast, the slower synaptic currents were almost completely blocked by bicuculline (94 ± 1% inhibition of the peak current with 10 μM bicuculline; n = 8 cells) and unaffected by kynurenic acid (6 ± 4% inhibition of the peak current with 1 mM kynurenic acid; n = 5 cells), thus identifying these responses as GABAergic. These inhibitory neurons tended to be flatter, with simpler dendritic trees. Because of the recent finding that GABA and glycine can be co-released by spinal-cord interneurons (Jonas P *et al*, Science 1998,

281:419-424), we considered the possibility that the inhibitory responses we were recording were mediated, in part, by glycine receptors. However, the control current was barely affected (4 ± 2% inhibition; $n = 6$ cells) by 100 nM strychnine, confirming that the inhibitory currents were entirely GABAergic.

The excitatory and inhibitory currents had essentially identical rise-times (see Table 1), and the peaks of the currents changed linearly with test potential (see insets to Fig. 1). The decay phase of the synaptic current $I(t)$, where t is the time measured from the peak of the current, was fit by a biexponential equation of the form

$$I(t) = I_{fast}\, e^{-t/\tau_{fast}} + I_{slow}\, e^{-t/\tau_{slow}}$$

where I_{fast} and I_{slow} are the amplitudes and τ_{fast} and τ_{slow} are the time constants of the fast and slow components, respectively. The values for these decay time constants measured from control excitatory and inhibitory responses are given in Table 1. In both cases, approximately two-thirds of the total charge transfer was carried by the slow component. For the excitatory glutamatergic responses, this slow component can be readily identified as being mediated by NMDA receptors because it is completely (99 ± 1%; n = 10 cells) blocked by 200 μM AP5 (DL-2-amino-5-phosphonopentanoate), a highly selective NMDA receptor antagonist (Davies J *et al*, Neurosci. Letts 1981, 21:77-81). In the presence of this concentration of AP5 the decay phase of the synaptic current could be fitted well by a single exponential with magnitude and time course little different to those of the control fast component. This fast component, which very largely determines the magnitude of the peak excitatory current ($I_{fast}/I_{total} = 92 \pm 1\%$; $n = 13$ cells), can be attributed to currents mediated by AMPA/KA receptors (Bekkers JM *et al*, Proc. Natl. Acad. Sci. U.S.A. 1991, 88:7834-7838; Mennerick S *et al*, J. Neurophysiol. 1995, 73:320-332).

The effects of xenon on synaptic currents

The gaseous concentration of xenon which prevents a response to a painful stimulus (*i.e.* MAC) appears to vary among species, being 71% atm in humans (Cullen SC *et al*, Anesthesiology 1969, 31:305-309), 98% atm in rhesus monkeys (Whitehurst SL *et al*, J. Neurosurg. Anesthesiol. 1994, 6:275-279) and 161% atm in rats (Koblin DD *et al*, Anesth. Analg. 1998, 87:419-424). When these values are converted to free aqueous concentrations at 37°C (Franks NP *et al*, Br. J. Anaesth. 1993, 71:65-76; Franks NP *et al*, Anesthesiology, 1996, 84:716-720) using an Ostwald water/gas partition coefficient of 0.0887 (Weathersby PK *et al*, Undersea Biomed. Res. 1980, 7:277-296), the values obtained are 2.5 mM, 3.4 mM and 5.6 mM for humans, monkeys and rats respectively, with the average value being 3.8 mM. For the experiments described herein, performed at room temperature, the concentration of xenon present in the standard test solution was 3.4 mM. At this concentration, xenon had negligible effects on the inhibitory synaptic currents but strongly depressed the excitatory currents. This is illustrated with representative traces in Figure 2.

For the GABAergic synaptic currents, 3.4 mM xenon affected neither the peak value, nor the time course of the postsynaptic currents. Percentage changes in the various inhibitory synaptic parameters are listed in Table 2, where it can be seen that none were significantly changed. The effects of xenon on currents evoked by a low (3 μM) concentration of exogenously applied GABA were also investigated. Here, 4.3 mM xenon had no significant effect on the GABA-induced current (2 ± 3% potentiation, n = 4 cells). A representative pair of traces is shown in the inset to Fig. 2A. In contrast, 3.4 mM xenon greatly depressed the glutamatergic synaptic current, with the effect being confined, almost exclusively, to the slow NMDA receptor-mediated component of the current (Fig. 2B). This is evident in the percentage changes in the various excitatory synaptic parameters which are listed in Table 2. Here it can be seen that the qualitative effects of xenon are remarkably closely mimicked by the effects of AP5. At the given concentration of AP5 (200 μM), the NMDA receptor component would be expected to be almost completely blocked (Davies J *et al*, Neurosci. Letts 1981, 21:77-

81), and this is consistent with the 99% block of I_{slow} (see Table 2). This is accompanied by a 75% reduction in total charge transfer, close to the 61% of the total charge which we estimate to be carried by the slow NMDA receptor-mediated component (see Table 1). The difference may be accounted for by the small, but significant reduction of the fast time constant τ_{fast} by AP5 (Table 2). Xenon, likewise, causes a large inhibition (70%) of I_{slow} and a large inhibition (56%) of the total charge transfer, with only a small effect on the fast AMPA/KA receptor-mediated component.

Neuroprotective Effect

Co-culturing neonatal glial and foetal neuronal cells

Dissociated murine cortical cell cultures were prepared using whole cerebral neocortices from fetal mice (14-17 days of gestation). Cortical glial cell cultures were prepared from early postnatal mice. After halting non-neuronal cell division with cytosine arabinoside the surviving cortical neuronal cells were plated onto the glial cell background and co-cultured.

Injuring neuronal co-culture with NMDA

15 days after originally harvesting the foetal cortical cells, the co-culture plates were exposed at room temperature to N-methyl,D-aspartate (NMDA), 10-1,000 μM, in a control salt solution (CCS) for 10 minutes. After carefully washing out with CCS, the cells were incubated for 6-24 hours in a Eagle's minimal essential medium free of glutamate or lactate dehydrogenase (LDH) but supplemented with glucose 25 mM, and $NaHCO_3$ 38 mM at 37°C in humidified CO_2 (5%) atmosphere. The degree of injury was determined by measuring the LDH released into the medium. A dose-response curve of NMDA-induced injury is shown in Figure 10, as described hereinbefore.

Injuring neuronal co-culture with glutamate

15 days after originally harvesting the cortical cells, the co-culture plates were exposed at room temperature to glutamate 10-1,000 μM, in CCS from 10-30 minutes. After carefully washing out with CCS, the cells were incubated for 6-24 hours in a Eagle's minimal essential medium free of glutamate or lactate dehydrogenase (LDH) but supplemented with glucose 25 mM, and $NaHCO_3$ 38 mM at 37°C in humidified CO_2 (5%) atmosphere. The degree of injury was determined by measuring the LDH released into the medium. A dose-response curve of glutamate-induced injury is shown in Figure 11, as described hereinbefore.

Protecting NMDA injured neuronal-glial co-cultured cells with xenon

75% of one atmosphere of xenon was delivered to the cells during and after exposure to a 750 μM NMDA, a concentration which produces a near maximal injury. The degree of injury was determined by measuring the LDH released into the medium (after correcting for "baseline" LDH release). Xenon reduced the injury produced by NMDA by more than 30 %; when xenon was continued after NMDA exposure, the injury was further reduced by more than 55 %. The results of the protective effect of xenon are illustrated in Figure 12, as described hereinbefore.

Protecting glutamate injured neuronal-glial co-cultured cells with xenon

75% of one atmosphere of xenon was delivered to the cells during and after exposure to 75 μM glutamate, a concentration which produces a near maximal injury. The degree of injury was determined by measuring the LDH released into the medium (after correcting for "baseline" LDH release). Xenon reduced the injury produced by glutamate by more than 35 %; when xenon was continued after glutamate exposure, the injury was further reduced by more than 60 %. The results of the protective effect of xenon are illustrated in Figure 13, as described hereinbefore.

Inhibition of synaptic plasticity

Perturbation of function in the central nervous system can be produced by a change in the strength of neural connections, which is referred to as synaptic plasticity. The present inventors have used the neuroblastoma X glioma hybrid cell line to examine the effect of xenon on synaptic plasticity. In this paradigm the alpha-2 adrenergic receptors, which are pivotally involved in synaptic transmission, inhibit adenylyl cyclase (AC).

To examine the inhibitory effect of alpha-2 adrenergic receptors on AC it is first necessary to stimulate the endogenous AC with Prostaglandin E_1 (PGE_1). Increasing doses of PGE_1 dose-dependently stimulates AC (Figure 14). When the alpha-2 agonist norepinephrine is co-administered under conditions in which AC activity is stimulated with PGE_1 , this activity is dose-dependently inhibited by norepinephrine (Figure 15).

To induce "synaptic plasticity" cells are pretreated for 8 hours with norepinephrine; after preparing homogenates from thoroughly washed cells, re-applied norepinephrine loses its inhibitory effect on PGE_1-stimulated AC activity. If these cells are co-incidently exposed to xenon during the pretreatment phase, the inhibitory action of norepinephrine will be sustained.

Modifications to the present invention will be apparent to those skilled in the art. The references mentioned herein are incorporated herein by reference.

Table 1: Control parameters for synaptic currents.

Parameter	Excitatory currents	n^a	Inhibitory currents	n^a
Time to peak (ms)	4.9 ± 0.6	12	5.0 ± 0.3	14
Decay half-time (ms)	7.8 ± 0.5	13	38.5 ± 3.5	16
τ_{fast} (ms)	9.0 ± 0.7	13	38.1 ± 3.9	16
τ_{slow} (ms)	210 ± 28	13	229 ± 44	16
I_{fast}/I_{total} [b]	0.92 ± 0.01	13	0.70 ± 0.04	17
Q_{slow}/Q_{total} [c]	0.61 ± 0.04	13	0.63 ± 0.04	16

[a] number of cells

[b] $I_{total} \equiv I_{fast} + I_{slow}$

[c] $Q_{total} \equiv Q_{fast} + Q_{slow}$

Table 2: The effects of 3.4 mM (~1 MAC) xenon on synaptic currents and of 200 μM AP5 on excitatory synaptic currents

	Inhibitory currents			Excitatory currents					
	+ 3.4 mM xenon			+ 3.4 mM xenon			+ 200 μM AP5		
Parameter	% change (means ± SEM)	↑, ↓ or ns[a]	n^b	% change (means ± SEM)	↑, ↓ or ns[a]	n^b	% change (means ± SEM)	↑, ↓ or ns[a]	n^b
I_{peak}	-3.1 ± 2.2	ns	11	-13.5 ± 1.8	↓	10	-16.3 ± 2.1	↓	10
Decay half-time	-3.4 ± 2.4	ns	10	-22.7 ± 2.5	↓	11	-24.1 ± 3.3	↓	10
Total charge transfer	-2.3 ± 3.9	ns	10	-55.6 ± 2.7	↓	11	-75.2 ± 2.5	↓	10
I_{fast}	-4.3 ± 2.8	ns	11	-4.1 ± 5.2	ns	11	6.8 ± 6.2	ns	10
I_{slow}	18.8 ± 12.4	ns	11	-70.3 ± 2.4	↓	11	-98.8 ± 0.8	↓	10
τ_{fast}	-1.3 ± 3.5	ns	11	-12.3 ± 1.8	↓	11	-5.9 ± 2.3	↓	10
τ_{slow}	0.8 ± 6.0	ns	11	-6.7 ± 6.5	ns	11	–	–	–
Q_{fast}	1.5 ± 7.2	ns	12	-11.4 ± 5.6	ns	11	5.3 ± 9.6	ns	10
Q_{slow}	12.6 ± 8.4	ns	12	-68.2 ± 4.2	↓	11	-96.0 ± 3.4	↓	10

[a]not significant at the 95% confidence level (Student's *t*-test), [b]number of cells

Claims

1. Use of xenon as a neuroprotectant and/or as an inhibitor of synaptic plasticity.

2. Use according to claim 1 wherein the xenon is an NMDA antagonist.

3. A process for the preparation of a pharmaceutical composition suitable for neuroprotection and/or inhibition of synaptic plasticity, which process comprises adding xenon to a pharmaceutically acceptable carrier, excipient or diluent, wherein the improvement comprises using xenon as a neuroprotectant and/or inhibitor of synaptic plasticity.

4. A pharmaceutical composition for neuroprotection and/or inhibition of synaptic plasticity which comprises xenon and a pharmaceutically acceptable carrier, excipient or diluent, wherein the improvement comprises using xenon as a neuroprotectant and/or inhibitor of synaptic plasticity.

5. Use of xenon in the preparation of a pharmaceutical for use in neuroprotection and/or inhibition of synaptic plasticity.

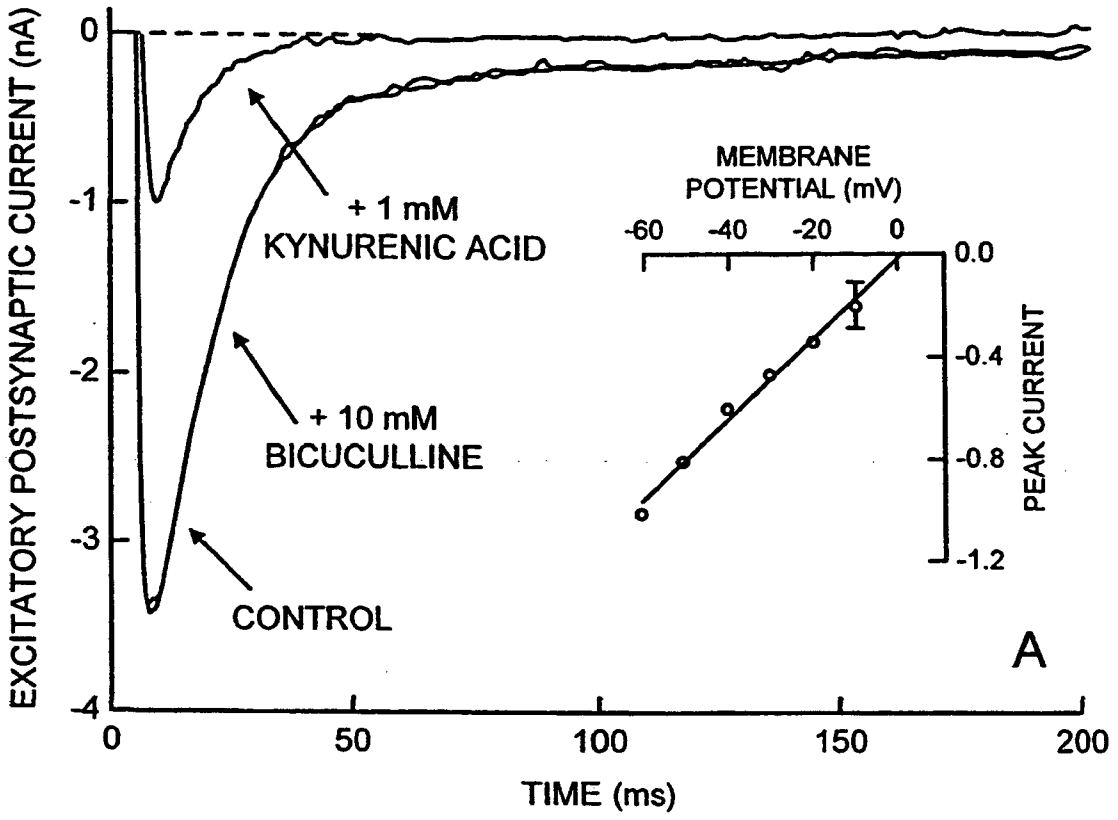
6. A use according to claim 10 wherein the xenon in the pharmaceutical is used in combination with a pharmaceutically acceptable carrier, diluent or excipient.

7. A use according to claim 10 wherein the pharmaceutical is for use in treating a condition associated with NMDA receptor activity.

8. A use according to claim 10 wherein the pharmaceutical is for use in treating a condition associated with NMDA receptor activation.

9. A use according to claim 10 wherein the pharmaceutical is for use in reducing the level of activation of the NMDA receptor.

10. The use of Xenon substantially as described in the examples.


EXCITATORY POSTSYNAPTIC CURRENT (nA)
0
-1
-2
-3
-4
+ 1 mM
KYNURENIC ACID
+ 10 mM
BICUCULLINE
CONTROL
MEMBRANE
POTENTIAL (mV)
-60 -40 -20 0
0.0
-0.4
-0.8
-1.2
PEAK CURRENT
A
0 50 100 150 200
TIME (ms)

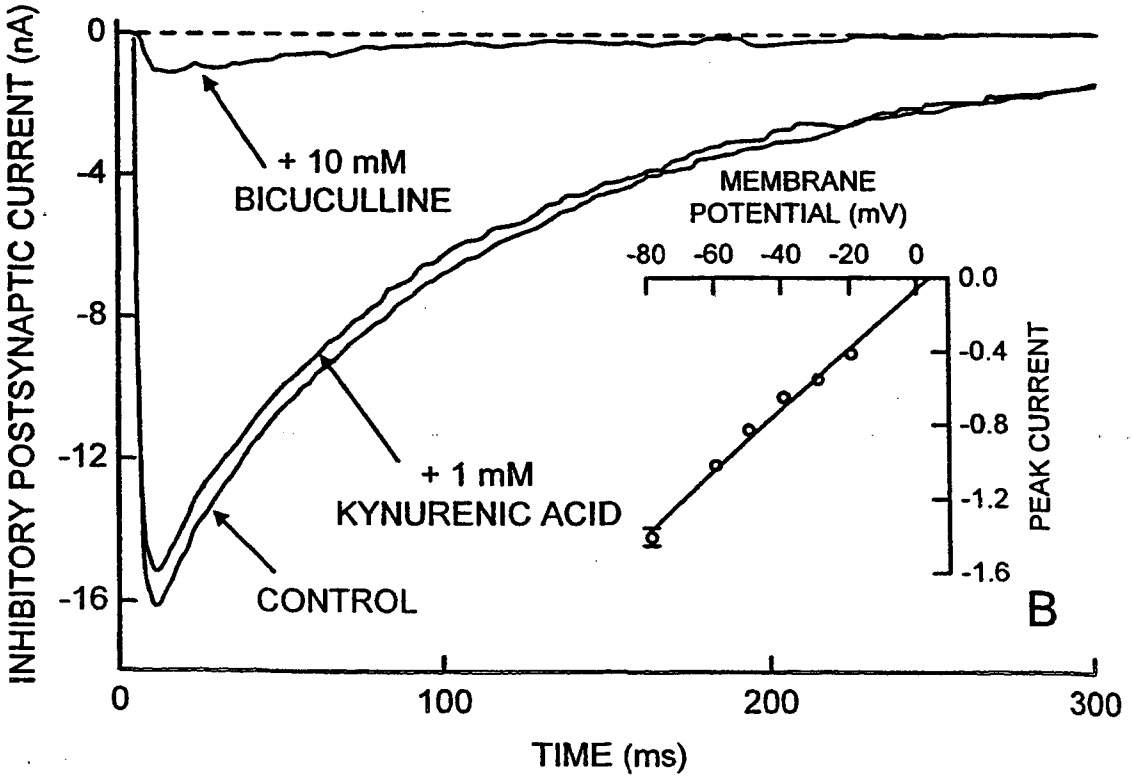

INHIBITORY POSTSYNAPTIC CURRENT (nA)
0
-4
-8
-12
-16
+ 10 mM
BICUCULLINE
+ 1 mM
KYNURENIC ACID
CONTROL
MEMBRANE
POTENTIAL (mV)
-80 -60 -40 -20 0
0.0
-0.4
-0.8
-1.2
-1.6
PEAK CURRENT
B
0 100 200 300
TIME (ms)

FIG. 1

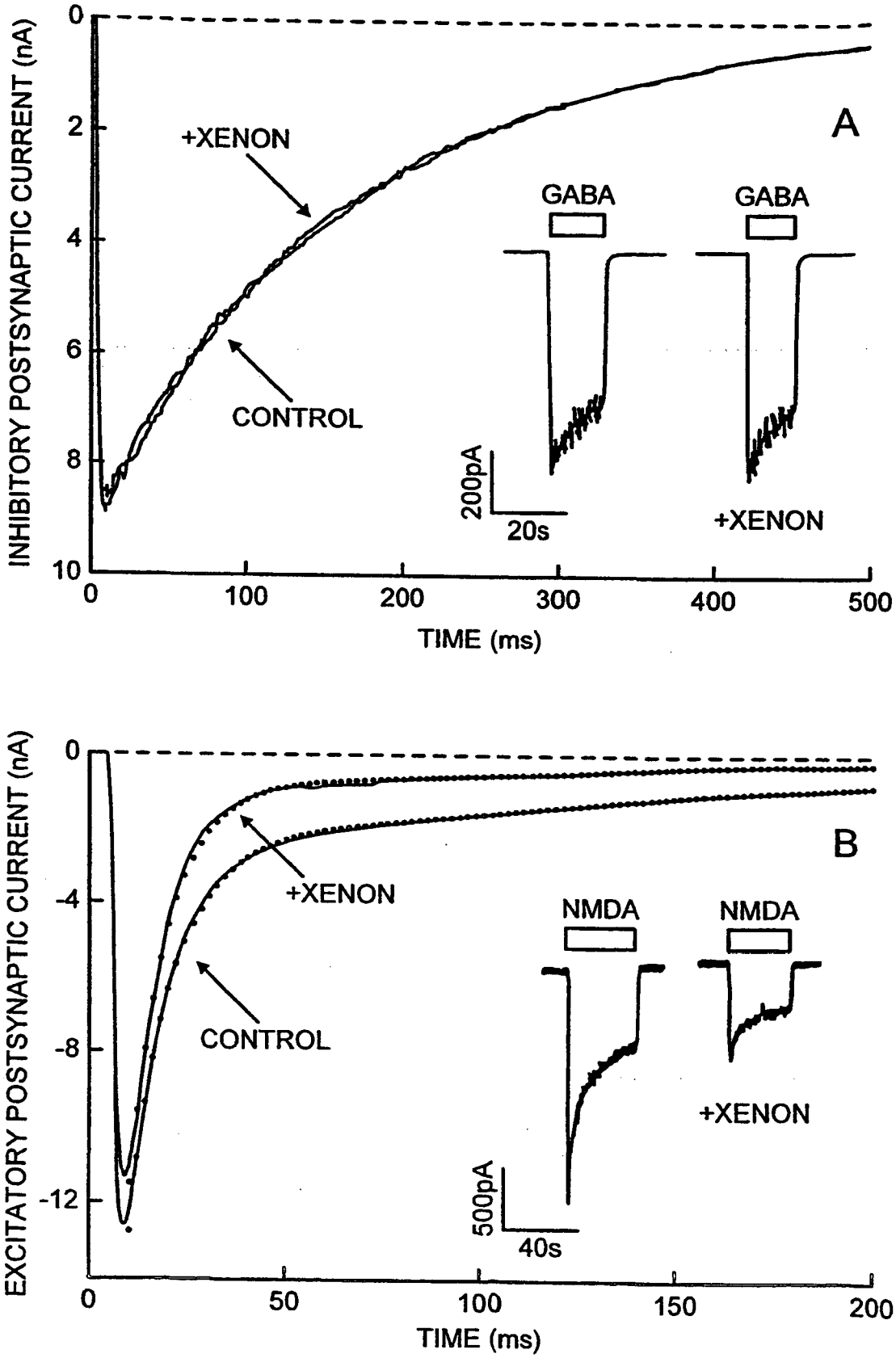

INHIBITORY POSTSYNAPTIC CURRENT (nA)
0
2
4
6
8
10
+XENON
CONTROL
A
GABA
GABA
200pA
20s
+XENON
0
100
200
300
400
500
TIME (ms)
EXCITATORY POSTSYNAPTIC CURRENT (nA)
0
-4
-8
-12
+XENON
CONTROL
B
NMDA
NMDA
500pA
40s
+XENON
0
50
100
150
200
TIME (ms)

FIG. 2

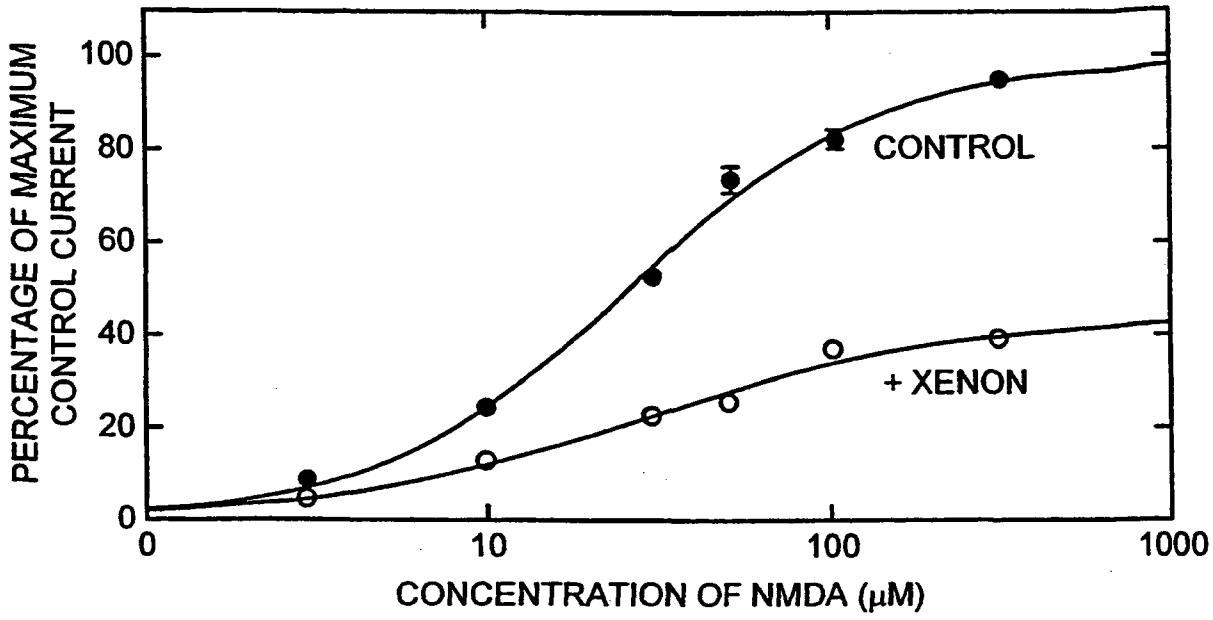

PERCENTAGE OF MAXIMUM CONTROL CURRENT
100
80
60
40
20
0
0
10
100
1000
CONTROL
+ XENON
CONCENTRATION OF NMDA (μM)

FIG. 3

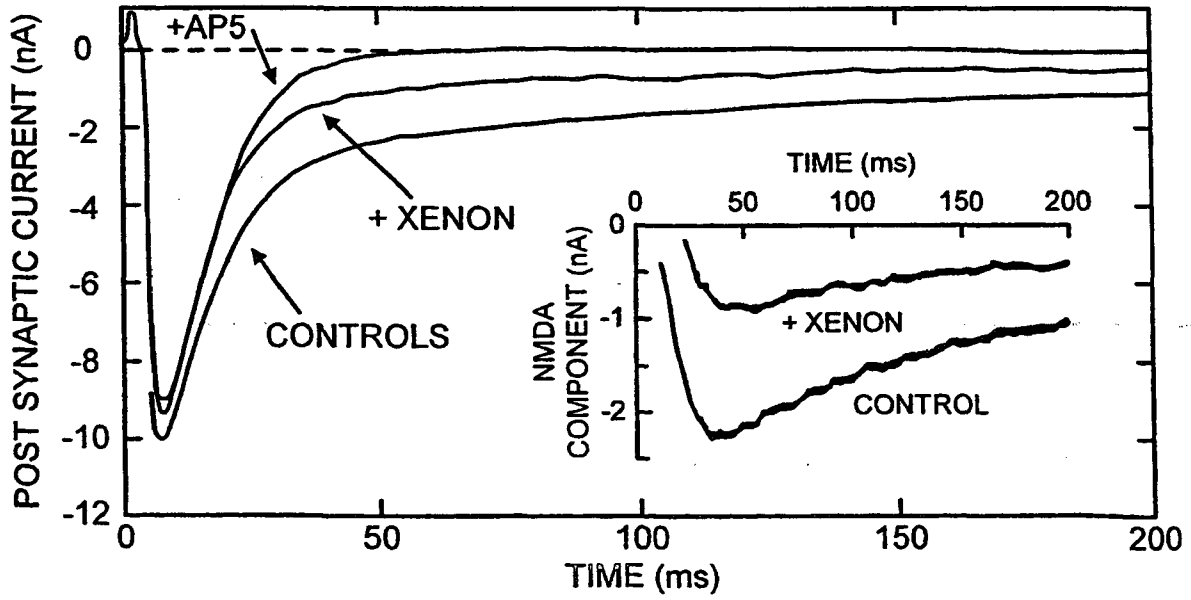

POST SYNAPTIC CURRENT (nA)
0
-2
-4
-6
-8
-10
-12
+AP5
+ XENON
CONTROLS
0
50
100
150
200
TIME (ms)
NMDA COMPONENT (nA)
0
-1
-2
TIME (ms)
0
50
100
150
200
+ XENON
CONTROL

FIG. 4

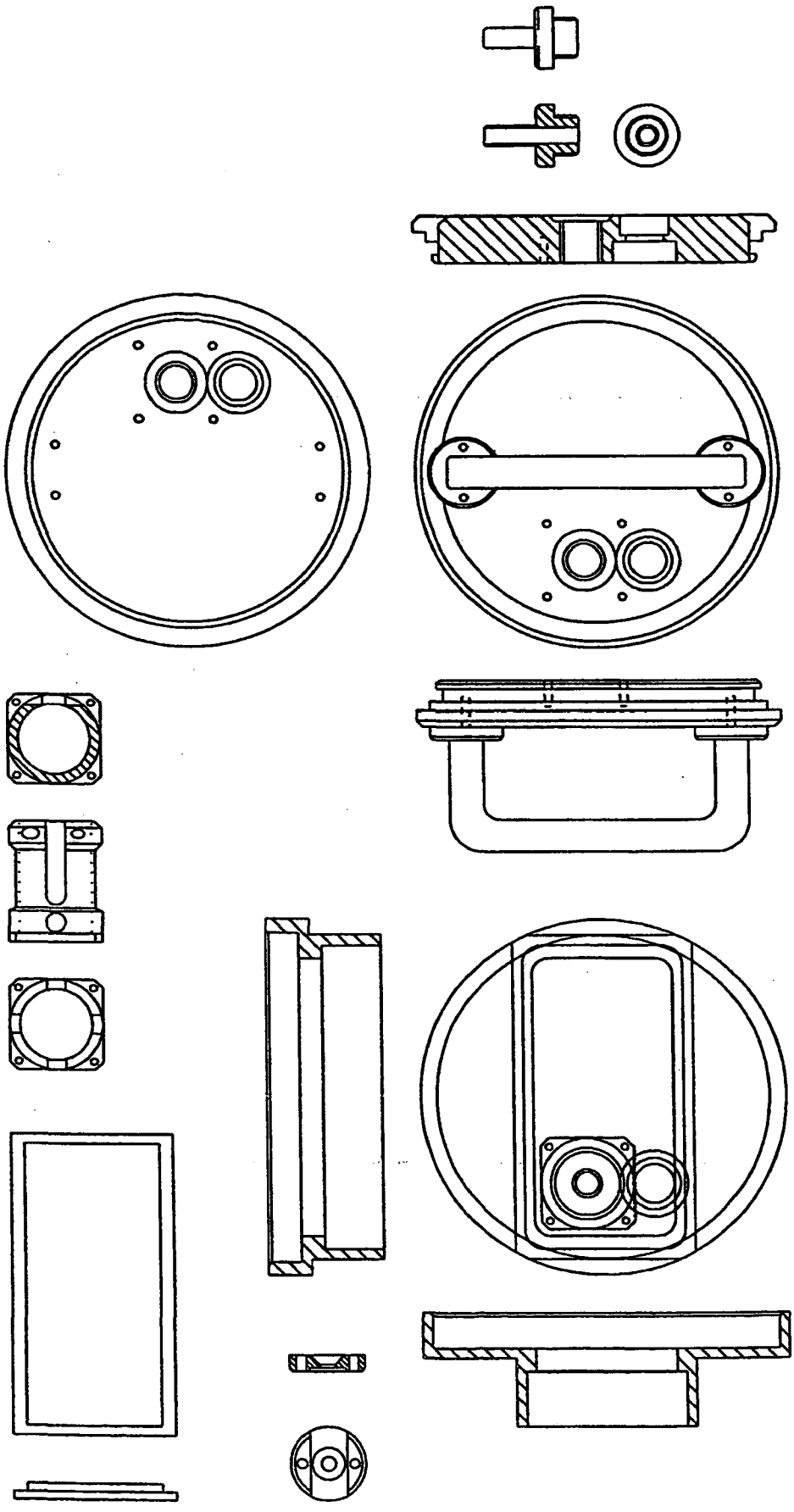


FIG. 5

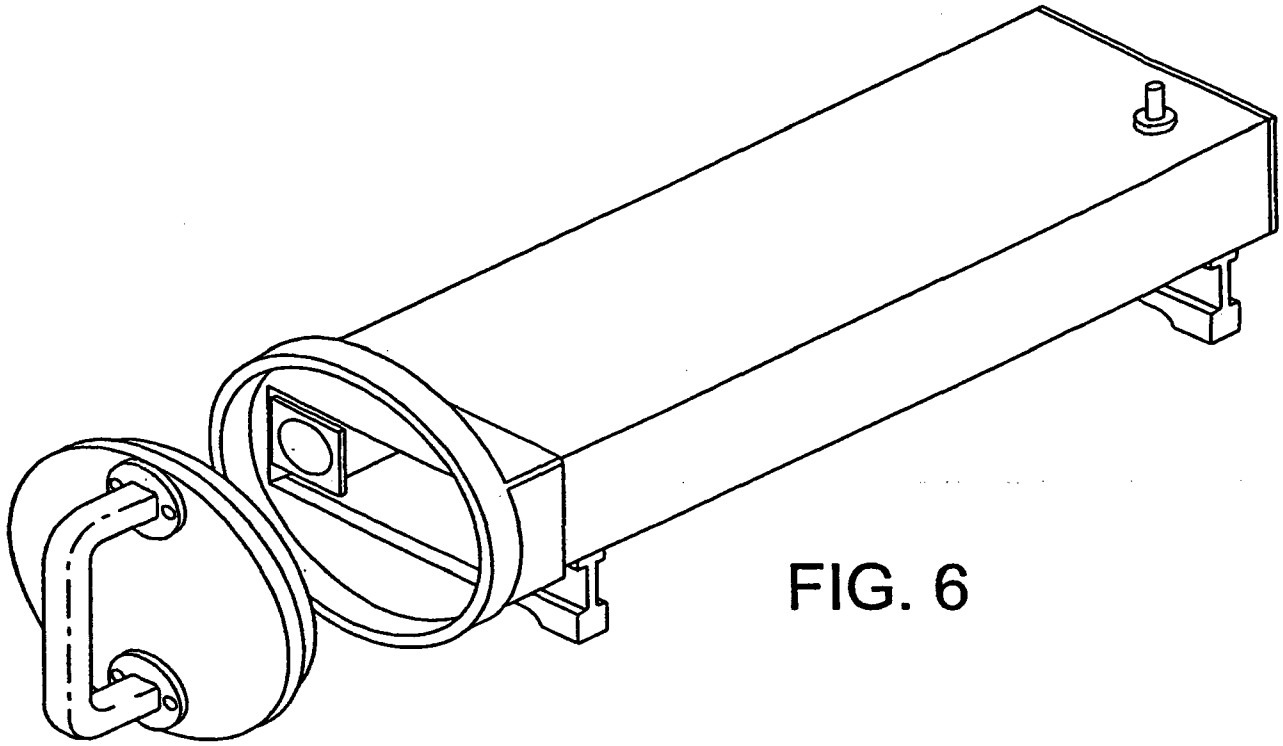


FIG. 6

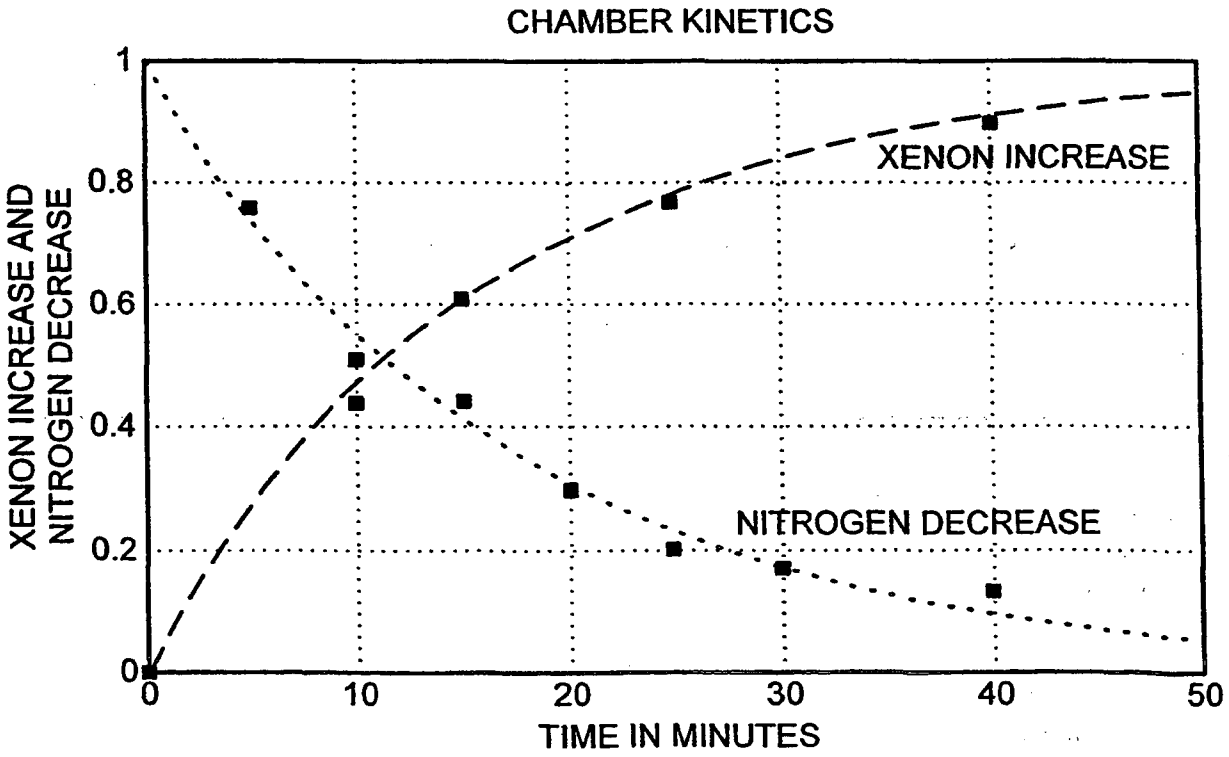

CHAMBER KINETICS
XENON INCREASE AND NITROGEN DECREASE
1
0.8
0.6
0.4
0.2
0
XENON INCREASE
NITROGEN DECREASE
0
10
20
30
40
50
TIME IN MINUTES

FIG. 7

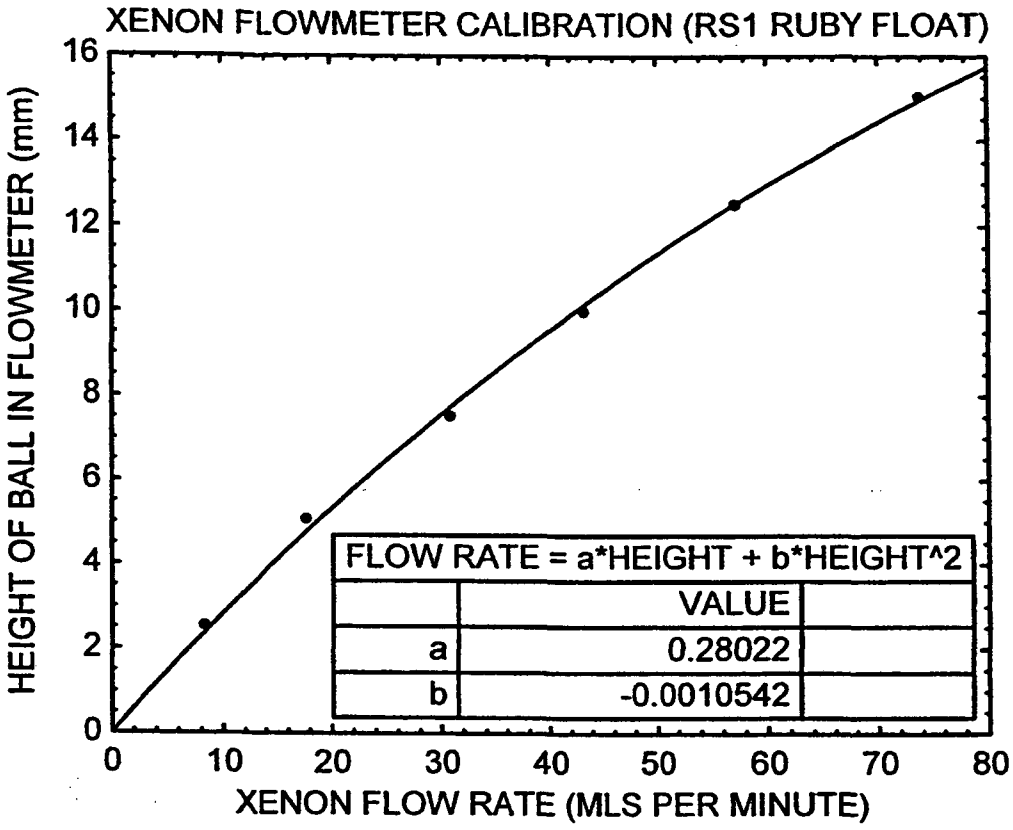

XENON FLOWMETER CALIBRATION (RS1 RUBY FLOAT)
HEIGHT OF BALL IN FLOWMETER (mm)
XENON FLOW RATE (MLS PER MINUTE)
FLOW RATE = a*HEIGHT + b*HEIGHT^2
VALUE
a 0.28022
b -0.0010542

FIG. 8

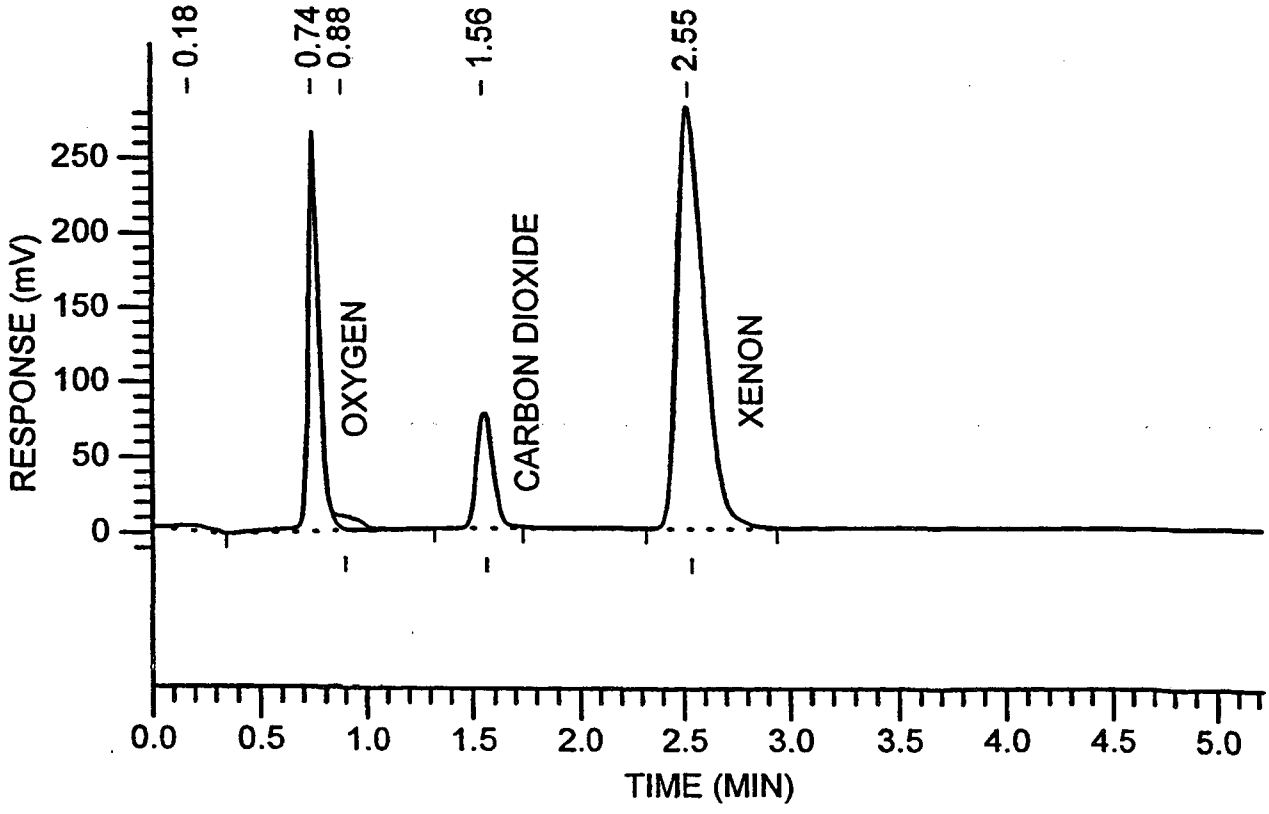

0.18
0.74
0.88
1.56
2.55
OXYGEN
CARBON DIOXIDE
XENON
RESPONSE (mV)
TIME (MIN)

FIG. 9

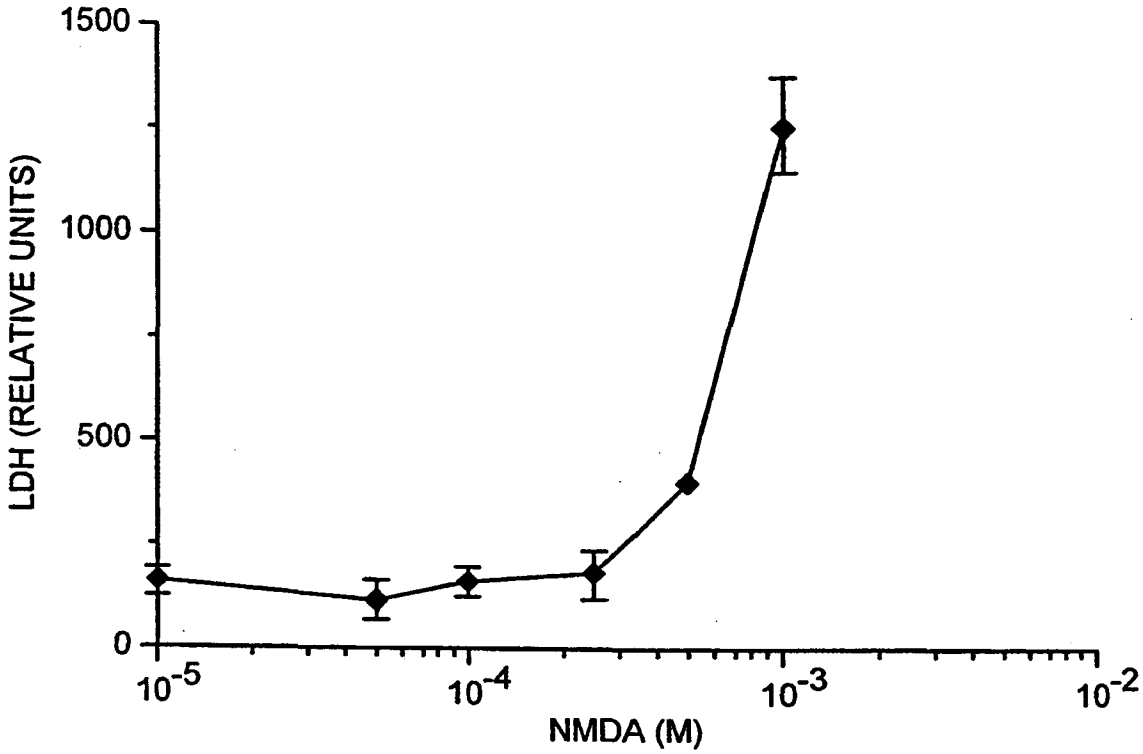

1500
1000
500
0
LDH (RELATIVE UNITS)
10^{-5}
10^{-4}
10^{-3}
10^{-2}
NMDA (M)

FIG. 10

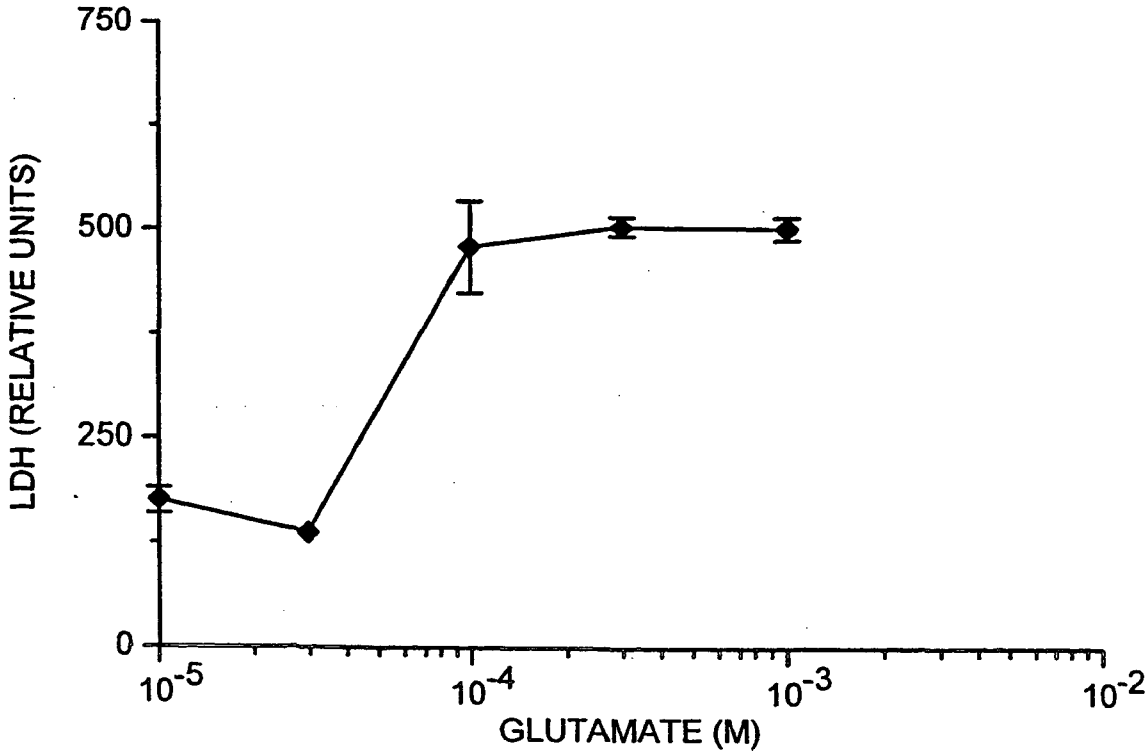

750
500
250
0
LDH (RELATIVE UNITS)
10^{-5}
10^{-4}
10^{-3}
10^{-2}
GLUTAMATE (M)

FIG. 11

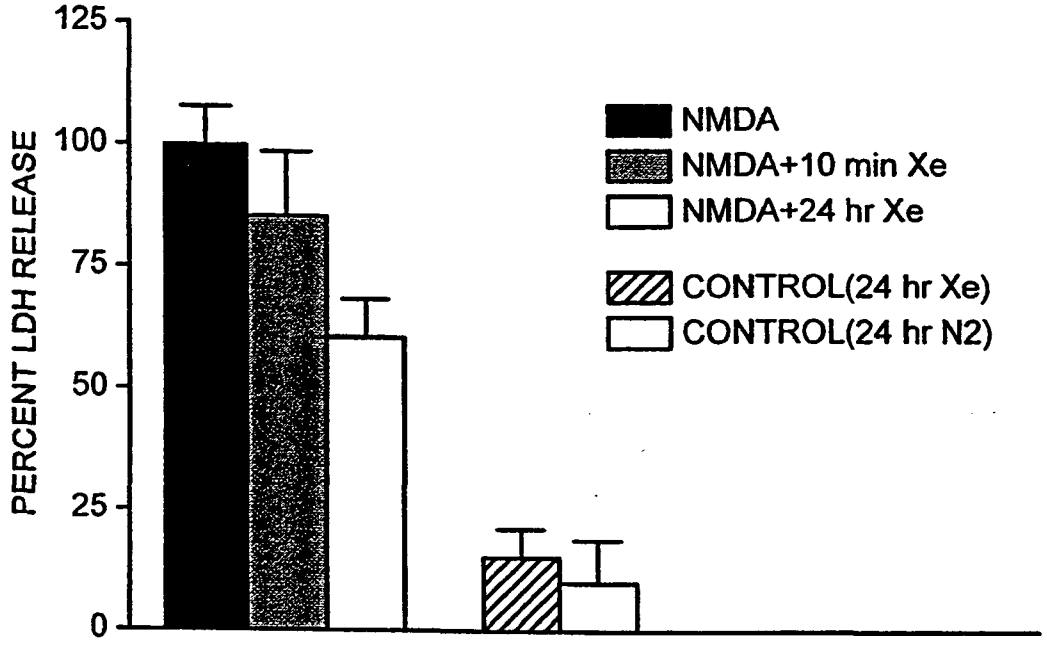

125
100
75
50
25
0
PERCENT LDH RELEASE
NMDA
NMDA+10 min Xe
NMDA+24 hr Xe
CONTROL(24 hr Xe)
CONTROL(24 hr N2)

FIG. 12

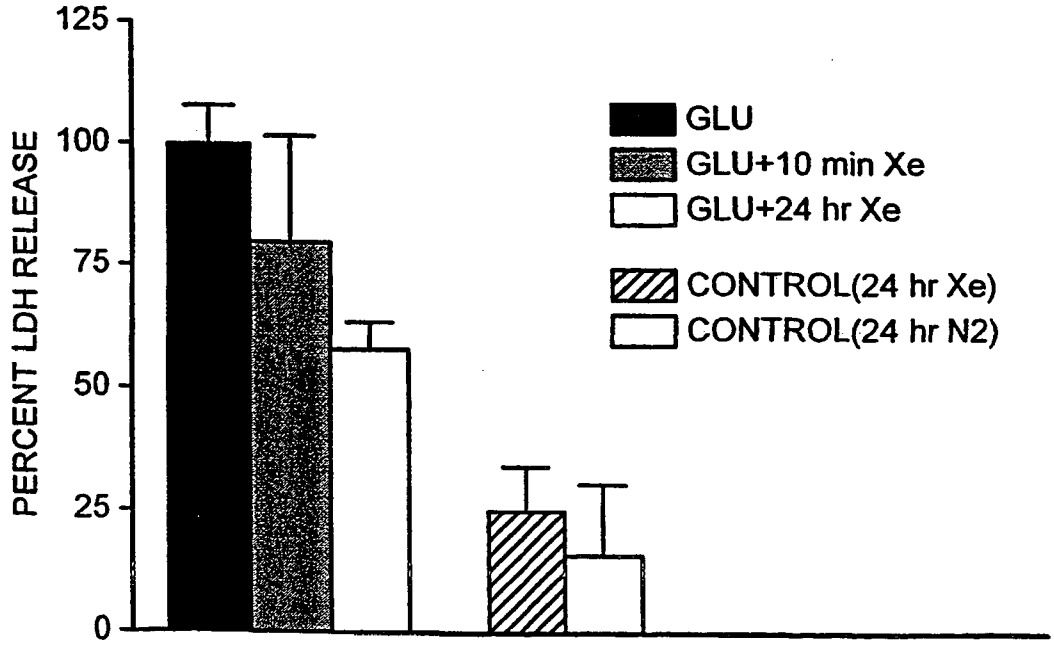

125
100
75
50
25
0
PERCENT LDH RELEASE
GLU
GLU+10 min Xe
GLU+24 hr Xe
CONTROL(24 hr Xe)
CONTROL(24 hr N2)

FIG. 13

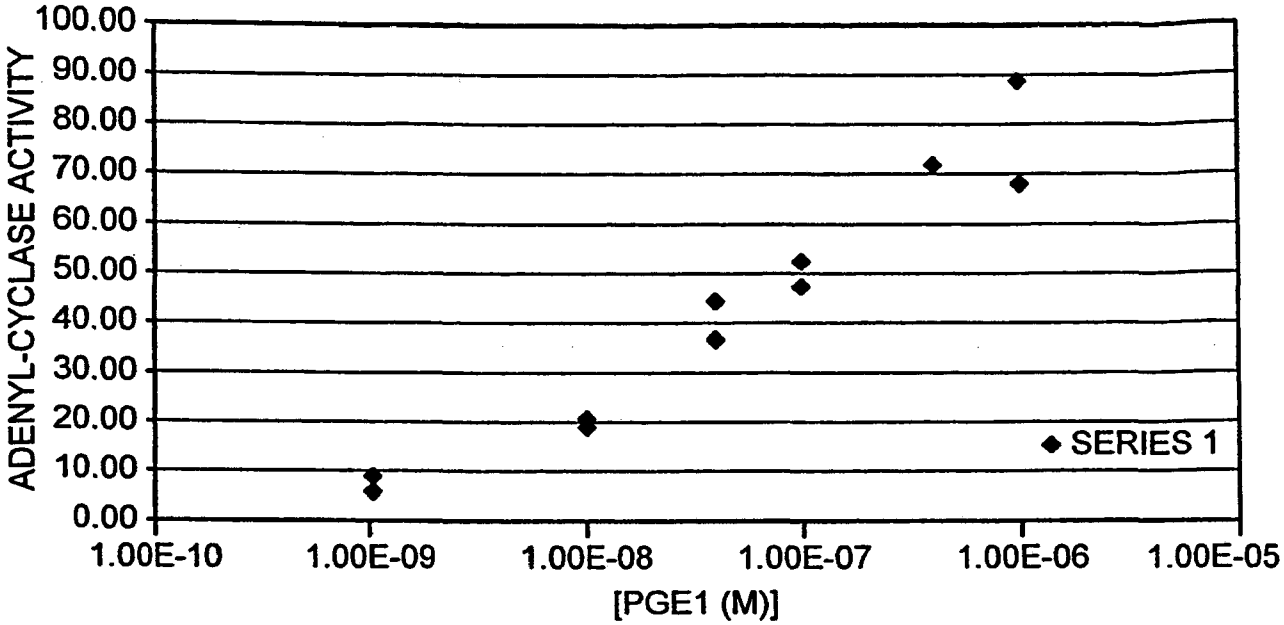

ADENYL-CYCLASE ACTIVITY
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
1.00E-10
1.00E-09
1.00E-08
1.00E-07
1.00E-06
1.00E-05
[PGE1 (M)]
SERIES 1

FIG. 14

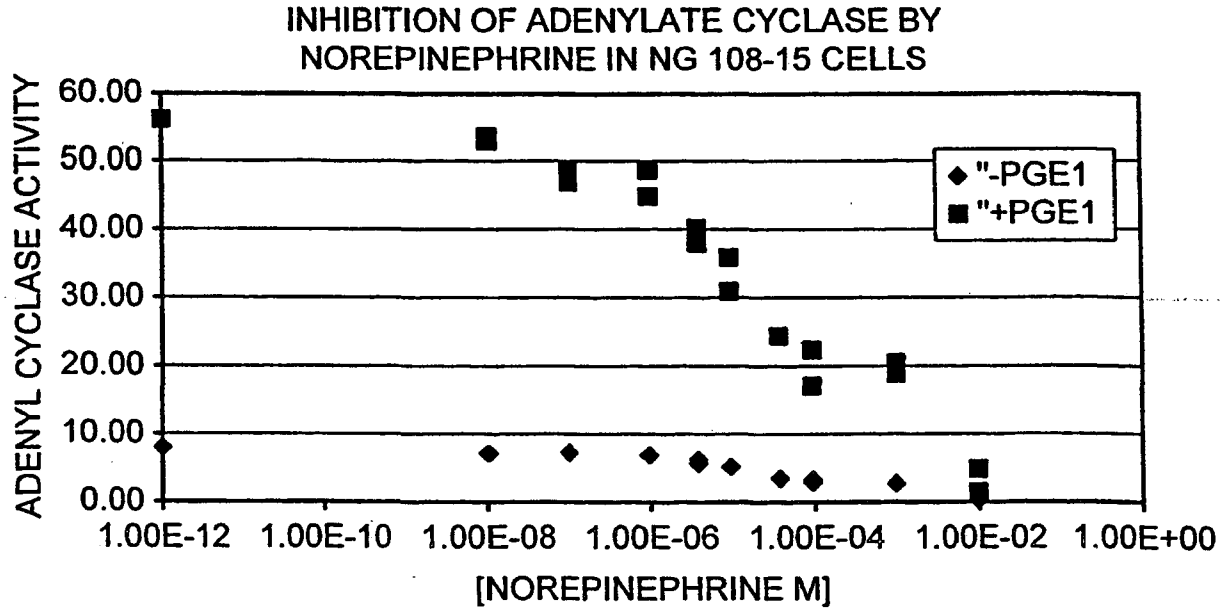

INHIBITION OF ADENYLATE CYCLASE BY
NOREPINEPHRINE IN NG 108-15 CELLS
ADENYL CYCLASE ACTIVITY
60.00
50.00
40.00
30.00
20.00
10.00
0.00
1.00E-12
1.00E-10
1.00E-08
1.00E-06
1.00E-04
1.00E-02
1.00E+00
[NOREPINEPHRINE M]
"-PGE1
"+PGE1

FIG. 15