Brain Research, 380 (1986) 186–190

Ketamine protects cultured neocortical neurons from hypoxic injury

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(Accepted April 22nd, 1986)

Key words: glutamate — ketamine — cyanide — cell culture — hypoxia — cell death

The general anesthetic ketamine, which has recently been reported to block the excitation of cortical neurons by N-methyl-D-aspartate (NMDA), was found to markedly reduce neuronal loss in murine neocortical cell cultures exposed to a hypoxic atmosphere or to cyanide. These observations may be relevant to attempts to find pharmacological means of minimizing hypoxic brain damage in the clinical setting.

It is not clear why the brain is so exquisitely sensitive to brief hypoxia, while other tissues may survive during hypoxia for extended periods. Recently, attention has been focused on a possible role of the excitatory neurotransmitter glutamate, or related compounds, in the pathogenesis of the neuronal injury seen with a variety of acute brain insults, including hypoxia. Glutamate is both present at high concentrations in the mammalian CNS[10] and toxic to central neurons[4]; if glutamate were abruptly released to the extracellular space, it is certainly possible that neuronal injury might result. Such an abrupt release of endogenous glutamate has been experimentally demonstrated from oxygen-deprived rat brain synaptosomes[5], as well as in vivo from rat hippocampus exposed to ischemia[2]. Further support for a role of glutamate in mediating hypoxic neuronal injury is the fact that certain glutamate antagonists can attenuate the acute neuronal injury produced not only by hypoxia[7], but also by ischemia[8] and by hypoglycemia[11].

The observed protective effects of glutamate antagonists on central neurons have raised the possibility that these drugs might have clinical therapeutic utility in hypoxic brain injury. Unfortunately, these drugs are not currently clinically available, and little is known of their systemic effects. Therefore recent reports that the clinically well-established general anesthetic drug, ketamine, appears also to have efficacy as a glutamate (and especially as a N-methyl-D-aspartate (NMDA)) antagonist, are of special interest[1,9]. We undertook the present study to determine if ketamine can protect cortical neurons from hypoxic injury, utilizing the simplified model system of dissociated murine neocortical cell culture, where the neuronal environment can be well controlled. We report here the novel finding that ketamine in fact substantially attenuates the cortical neuronal injury produced by either oxygen deprivation, or brief exposure to cyanide.

Dissociated murine cortical cell cultures were prepared as previously described[3], and used between 15 and 23 days after plating. Neuronal cultures were exposed to 12–32 h of hypoxia at 37 °C using the GasPak anerobic incubator (Becton Dickinson), a system which replaces atmospheric oxygen with 4–7% carbon dioxide, hydrogen, and water vapor. Immediately prior to hypoxic incubation, neuronal culture medium was replaced with Eagle's Minimal Essential Medium (MEM) (Earle's salts) with or without the addition of 1 mM ketamine. Other cortical neuronal cultures were exposed to a 20 min pulse of sodium cyanide (NaCN) at room temperature, in a Tris-buf-

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fered solution (composition in mM: NaCl 120, KCl 5.4, $MgCl_2$ 0.8, $CaCl_2$ 1.8, Tris-HCl 25, glucose 15) with or without 1 mM ketamine. Cyanide exposure was terminated by triple exchange with salt solution lacking Ca and Na (the latter replaced by choline), followed by incubation in this solution for 15 min. Cultures were then washed by double exchange with salt solution, returned to culture medium, and placed in the incubator overnight.

Individual microscope fields (200×) were photographed both before and after hypoxic injury (the latter both with phase-contrast, and with bright-field following a 5 min incubation in 0.4% Trypan blue dye), using an objective marker to assist relocation. Previous work[3] has established that neurons in these cultures can be easily identified by morphological criteria (large, phase-bright cell bodies and associated processes). For quantitative counts, only neurons identified unambiguously on the baseline photograph were counted; these identified neurons were later

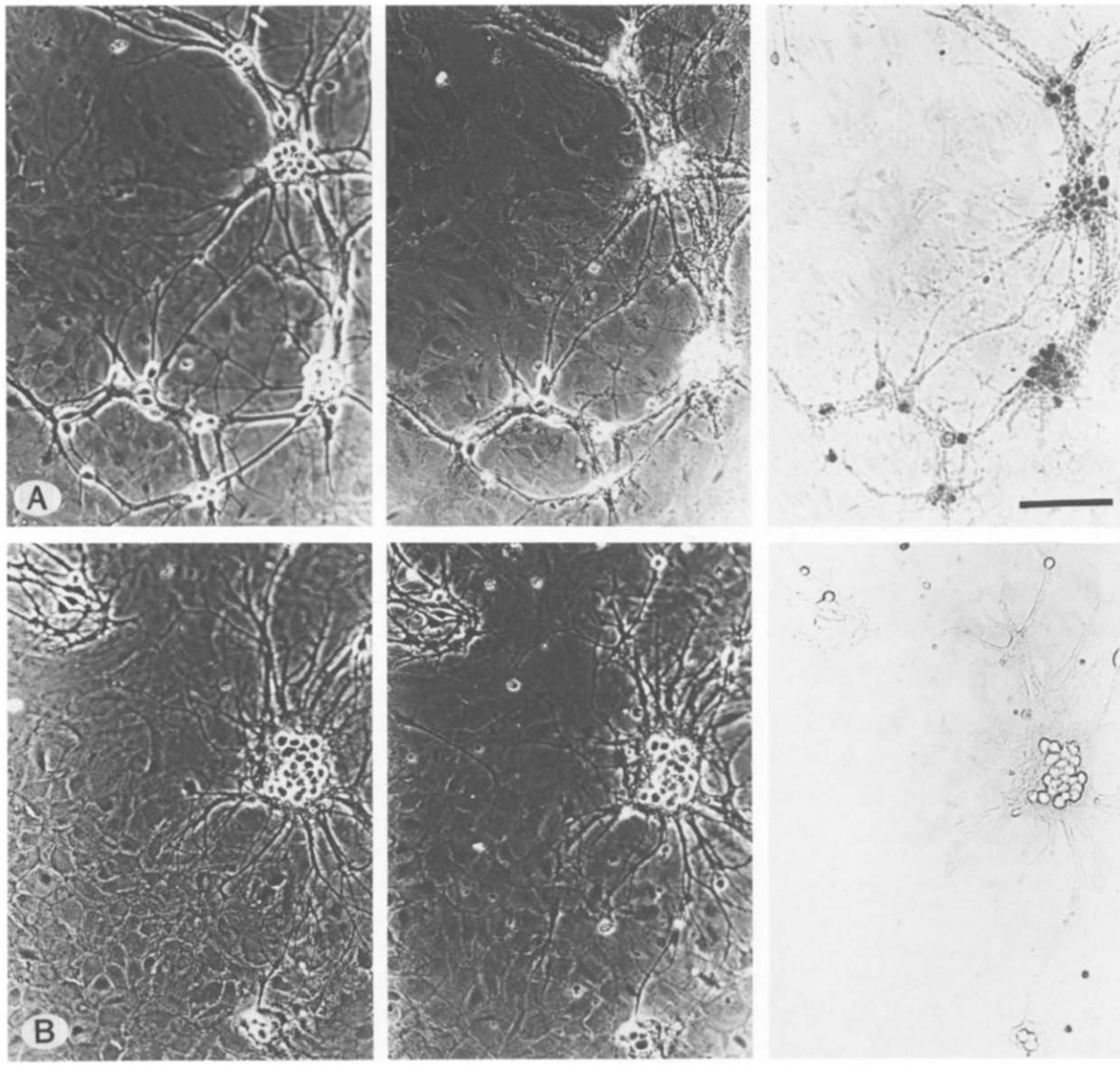

Fig. 1. A: photomicrographs of an identified field of cortical neurons, taken before (left: phase-contrast), and 20 h after oxygen deprivation (middle: phase-contrast, right: bright-field following 5 min incubation in 0.4% Trypan blue dye). B: same as A, except that the hypoxic exposure was carried out in the presence of 1 mM ketamine. Bar = 100 μm.

scored as lost if either absent (replaced by debris), or stained with trypan blue, on the follow-up photograph.

In untreated (control) cultures after 12–16 h of oxygen deprivation, neuronal bodies and processes appeared granular and irregular; some neurons were markedly swollen. After 20 h, most neurons stained with trypan blue dye, and many were replaced by debris (Fig. 1A). The background mat of non-neuronal cells (which are immunoreactive for glial fibrillary acidic protein, and hence are likely astrocytes — unpublished data), appeared intact. In contrast, neurons in cultures exposed to the same degree of hypoxia in the presence of 1 mM ketamine appeared remarkably preserved and excluded Trypan blue dye (Fig. 1B; 4 experiments); these neurons remained morphologically stable for at least another day after the exposure. The protective effect of ketamine was further verified by neuronal cell counts (Table I).

Control cortical cell cultures exposed to 20 mM cyanide showed reproducible marked swelling of the neuronal cell bodies and granularity of the processes that appeared after about 17 min of incubation (Fig. 2A). (With cyanide concentrations below 20 mM the appearance of this swelling was progressively delayed, and longer cyanide exposure was required for neuronal injury.) The following day, most neurons were replaced by debris (Fig. 2A). In contrast, neurons in cultures exposed to cyanide in the presence of 1 mM ketamine consistently failed to exhibit acute swelling in exposures of up to 40 min (8 experiments), and showed markedly less damage on restudy the following day (Fig. 2B; Table I) (4 experiments).

We used two empirically derived methods to produce hypoxic injury in neocortical cell cultures: environmental oxygen deprivation, and pulse exposure to a high concentration of cyanide. Cyanide blocks cellular respiration by direct inhibition of electron transport[6]; the greater rapidity of cyanide-induced neuronal injury compared with that produced by environmental oxygen deprivation may possibly be accounted for by the presence of residual media oxygen in the latter condition. The fact that the rapidity of cyanide-induced neuronal injury was dose dependent with pulses of cyanide in the millimolar range is a surprising finding given the high affinity of cyanide for its target binding site, cytochrome c oxidase, (ibid.), but one that might be accounted for by factors governing access to that binding site.

Addition of 1 mM ketamine prior to the hypoxic insult blocked the acute swelling seen immediately after cyanide exposure, and markedly improved neuronal survival following either cyanide exposure or prolonged oxygen deprivation. A similar protective effect of ketamine has recently been observed by Rothman in rat hippocampal cultures exposed to hypoxia (personal communication). In the context of evidence that certain excitatory amino acid receptor antagonists can reduce neuronal vulnerability to hypoxia and other acute insults (see above), it is attractive to speculate that the observed protective effects of ketamine are due to receptor level antagonism of the neurotoxic effect of endogenous glutamate (or related compounds), released in the setting of hypoxia. Furthermore, since ketamine appears to be a selective antagonist at the NMDA subclass of glutamate receptors (see above), exploration of the possibility that this subclass of receptors is particularly involved in hypoxic neuronal loss appears to be warranted. The possibility that some part of the protective effect of ketamine is mediated by actions unrelated to NMDA receptors, for example on neuronal metabolic rate or glial function, cannot be excluded by the present study.

Our results suggest that ketamine substantially reduces the vulnerability of cortical neurons in mixed cell cultures to hypoxic injury. If ketamine has a similar effect on neocortical neurons in vivo, it may prove to be therapeutically useful in a variety of disease states.

TABLE I

Effects of ketamine on hypoxic neuronal loss

n refers to the number of identified 200× fields counted in a single experiment; each field contained 26–106 neurons which could be unambiguously identified. Values are means ± S.D.

Condition	*Treatment*	*Cell loss (%)*
Oxygen deprivation	Control	83 ± 25
	Ketamine	5 ± 5 $P < 0.01$ (n = 3)*
Cyanide	Control	100 ± 0
	Ketamine	4 ± 3 $P < 0.001$ (n = 2)

* Two-tailed *t*-test.

We thank D. Bart for helpful discussions, and M.

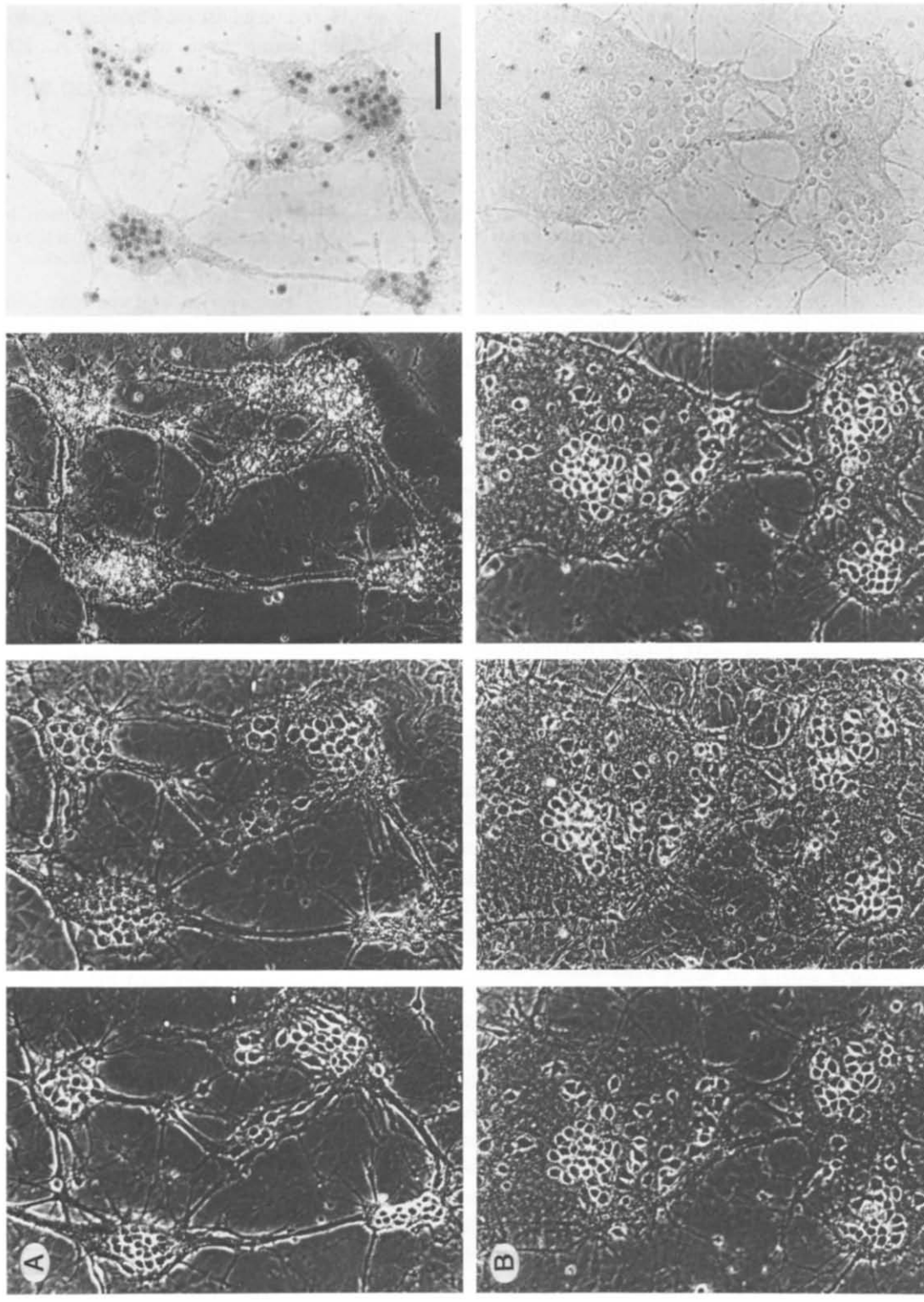

Fig. 2. A: photomicrographs of an identified field of cortical neurons, taken before (left: phase-contrast), immediately after (left middle: phase-contrast), and 1 day after a 20 min pulse exposure to 20 mM cyanide (right middle: phase-contrast, right, bright-field following incubation in Trypan blue dye). B: same as A, except that the cyanide exposure was carried out in the presence of 1 mM ketamine. Bar = 100 μm.

Yokoyama for expert assistance with cell cultures. Ketamine was a kind gift from Warner-Lambert. This research was supported by Grant NS21628 from the National Institute of Health, grants from the Wills and Hereditary Disease Foundations, and by a Hartford Fellowship from the John A. Hartford Foundation (to D.W.C.).

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