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Respiratory effects of glutamate receptor antagonists in neonate and adult mammals

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Abstract

We determined the conditions (immaturity, species, anesthesia, receptor blockade selectivity) under which glutamate receptor blockade produces respiratory depression in mammals. In unrestrained 0- to 2-day-old neonate and adult mice and cats, ventilation was measured by the barometric method, before and after separate or sequential administration of a non-NMDA receptor antagonist, NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(F)quinoxaline, 2–200 mg kg^{-1} in mice, 10–40 mg kg^{-1} in cats), and a NMDA receptor antagonist, dizocilpine (3 mg kg^{-1} in mice, 0.15–1.0 mg kg^{-1} in cats). NBQX or dizocilpine alone did not decrease ventilation in awake adults, but NBQX strongly depressed ventilation in neonate awake mice and in adult anesthetized animals. Given together, dizocilpine and NBQX always profoundly depressed ventilation by producing a lethal apnea in neonate mice, and an apneustic pattern of breathing in adults of both species and in neonate cats. We conclude that blockade of either NMDA or non-NMDA receptors is innocuous in awake adults. The factors which may potentiate respiratory depression are (1) anesthesia, (2) immaturity, and (3) combined blockade of both receptors types. The mechanism of depression is species-dependent and age-dependent.

Keywords: Respiration; NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(F)quinoxaline); Dizocilpine; Neonate; Development; (Cat); (Mouse)

1. Introduction

There is good evidence that an excitatory amino acid, probably glutamate, is the main neurotransmitter involved in fast excitatory connections within the network of respiratory neurons, which generates the respiratory rhythm in the mammalian brainstem, and drives motoneurons that command respiratory muscles (reviews in Bianchi et al., 1995; Bonham, 1995; Denavit-Saubié and Foutz, 1996). Combined administration of *N*-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists to adult cats produces an often lethal respiratory arrest in the inspiratory phase (apneusis) (Foutz et al., 1994; McManigle et al., 1994). This suggests that the combined use of these antagonists for the treatment of cerebral ischemia and other disorders (Gill, 1994) might produce hazardous side-effects on respiratory function. However, most studies concerning neuroprotective effects of glutamate receptor antagonists are performed in adult rodents, whereas most studies concerning their respiratory effects are performed in neonate rodents in vitro. Furthermore, conflicting results are reported in the literature concerning the effects of glutamate receptor antagonists on respiratory amplitude and frequency. Competitive antagonists of non-NMDA receptors such as NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(F)quinoxaline) (Sheardown et al., 1990) dose dependently decrease and suppress the respiratory motor output in various preparations from neonates in vitro (Liu et al., 1990; Greer et al., 1991; Funk et al., 1993) or adults in vivo (Pierrefiche et al., 1994), but they do not depress ventilation in intact spontaneously breathing cats (Foutz et al., 1994). Antagonists acting at NMDA receptors, such as dizocilpine (MK-801), do not alter respiratory motor output in neonate preparations in vitro (Liu et al., 1990; Greer et al., 1991), but decrease inspiratory amplitude in adult cats and rats (Foutz et al., 1988b, 1989; Connelly et al., 1992; Abrahams et al., 1993). The discrepancies are even greater concerning the effects of glutamate receptor antagonists on respiratory timing mechanisms. Non-NMDA receptor antagonists decrease respiratory fre-

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quency in neonate rodent preparations in vitro (Greer et al., 1991), but not in adult cats in vivo (Pierrefiche et al., 1994). NMDA receptor antagonists do not affect the timing of respiratory phases in neonates in vitro (Greer et al., 1991), but produce an extreme prolongation of the inspiratory phase in adult vagotomized animals (Foutz et al., 1988a). This latter discrepancy might be a consequence of in vitro vs. in vivo conditions because, with the same adult animal, NMDA receptor blockade prolongs the inspiratory phase in vivo, but becomes ineffective after isolation of the brainstem in vitro (Morin-Surun et al., 1995). However, most other discrepancies might be due to the important post-natal maturational changes that affect the respiratory network (Paton and Richter, 1995).

To test whether the effects of glutamate receptor antagonists on respiratory function are influenced by factors such as post-natal maturation, species differences, anesthetics, and receptor selectivity of the blockade, we administered non-NMDA and NMDA receptor antagonists, alone and in combination, to unrestrained adult and neonate cats and mice, and we measured respiratory activity by means of the non-invasive plethysmographic technique.

2. Materials and methods

2.1. Subjects

The experiments were performed with 17 kittens aged 0–2 days (body weight (b.wt) 123 g $\pm$ 10 g S.D.), 10 adult cats (b.wt 2.6 kg $\pm$ 0.5 kg), 39 swiss mouse pups aged 0–2 days (b.wt 1.9 $\pm$ 0.3 g), 2 mouse pups aged 7 days, 16 mouse pups aged 9–10 days (b.wt 7.0 $\pm$ 1.9 g) and 20 adult swiss mice (b.wt 25.0 $\pm$ 4.4 g). In each protocol involving neonates, pups from at least two different litters were used. All experiments were conducted in accordance with the regulations of the French Ministry of Agriculture.

2.2. Surgical procedures

Sterile procedures and pentobarbital anesthesia (35 mg kg^{-1} i.p.) were used to instrument adult cats as previously described (Foutz et al., 1988b). Permanent electrodes were implanted in the skull for recording electrocorticographic patterns. A silastic catheter, inserted into the right jugular vein for subsequent injection of drugs, led to a capped Luer needle embedded in an acrylic mound fixed to the skull. After surgery, at least one week was allowed for recovery before the first experiment, and between two successive experiments. No surgery was performed with kittens and mice.

2.3. Recording procedures

Respiratory activity was measured with the barometric method (Bartlett and Tenney, 1970). The animals were placed in a sealed plethysmograph chamber made of Plexiglas, which was periodically flushed with humidified air or hyperoxic gas mixtures. To alleviate possible hypoxemia due to respiratory depression, cats and adult mice inhaled a hyperoxic mixture (50% O_2, 50% N_2), changed to 100% O_2 during periods of apneusis. It has been shown that oxygen breathing does not modify ventilation in conscious cats (Gautier et al., 1986). Mouse pups inhaled room air throughout the experiment and were never given extra oxygen because the respiratory system may be more resistant to hypoxemia in neonate than in adult rodents (Ballanyi et al., 1996). The volume of the recording chamber was 25 l for recordings of adult cats, 2.4 l for kittens and 1.7 l for adult mice. For recording neonate mice, the plethysmograph chamber was an adapted 50 ml plastic syringe with the volume set at 20 ml. The chamber was connected to a reference box of the same size. The pressure difference between both chambers was measured by means of Validyne models DP 45-14 or DP 103-12 differential pressure transducers, and model CD15 carrier demodulator. The amplified spirogram was displayed on a rectilinear electrostatic recorder (Gould ES 1000) at speeds of 10 to 100 mm s^{-1} and/or digitized for off-line analysis (2-min records) on a PC-compatible microcomputer (adaptation of PCLAMP program, Axon Instruments). For each breath, the inspiratory (T_i) and expiratory (T_e) durations and tidal volume (V_T) were derived from the pressure signal (Bartlett and Tenney, 1970). We calculated the inspiratory fraction of the respiratory cycle (T_i/T_{tot}, with $T_{tot} = T_i + T_e$), respiratory frequency F (breaths/min), and minute volume (V_E). Ten to 200 successive breaths were averaged for each sample, depending on the species and pattern of breathing.

After the animal had become accustomed to the chamber, baseline data were collected during states of immobility (quiet waking or sleep), then the drug or vehicle were infused through the implanted catheter in adult cats, or injected i.p. (in kittens and adult mice) or s.c. (in neonate mice). We administered doses of NBQX higher than doses that had been shown to block at least 80% of the response of respiratory neurons to iontophoretic applications of a non-NMDA receptor agonist, and doses of dizocilpine (to mice and neonates) equal to or higher than doses that block completely the response of respiratory neurons to NMDA (see Section 4). Such 'saturating' doses should allow qualitative comparisons of effects between mature and neonate animals despite possible differences in bioavailability. For i.v. administration, NBQX (10 or 20 mg kg^{-1}) was dissolved in a 5% glucose solution (pH 8.4–8.7) and infused at a rate of 0.6 mg kg^{-1} min^{-1} (0.6–1.0 ml min^{-1}). For i.p. and s.c. administration, NBQX (2–200 mg kg^{-1}) was dissolved in a volume of 5% or 2.5% glucose not exceeding 15 μl g^{-1} b.wt Dizocilpine, a non-competitive NMDA channel blocker (Wong et al., 1986), was dissolved in saline and injected through the same routes as NBQX in volumes not exceeding 10 μl

g^{-1} b.wt (s.c. or i.p.), or 1–2 ml kg^{-1} (injected i.v. over 10–20 s). Pentobarbital was dissolved in saline and injected i.v. over 1–2 min. Body temperature was measured by means of a thermocouple thermometer (Digisense). A small flexible rectal probe (diameter 0.6 mm) was inserted at the end of the control session and left in place. In mouse pups, oral temperature was measured before and after each 2-min recording period.

2.4. Pharmacokinetics of NBQX in the cat

Unlike dizocilpine, which has a long plasma half-life ($t_{1/2}$) in all species studied (Hucker et al., 1983), and affects respiratory function in cats for more than 10 h after a single bolus dose of 0.3–1.0 mg kg^{-1} (Foutz et al., 1989), NBQX has a relatively short half-life in rats ($t_{1/2} <$ 1 h), and a longer one in mice (1–4 h) (Dalgaard et al., 1994). Since no data were available for cats, we determined the plasma $t_{1/2}$ in five of the permanently instrumented cats. Under pentobarbital anesthesia (35 mg kg^{-1}), a small catheter was inserted into a leg vein, and a slow NBQX infusion was started (0.6–0.8 mg kg^{-1} min^{-1}) to a final dose of 10 mg kg^{-1} ($n = 4$) or 20 mg kg^{-1} ($n = 1$). Following the infusion period, blood samples (1.5 ml) were collected through the jugular catheter every 10 min for 60 min, then every 20 min for 60 min. Plasma was frozen and stored. The NBQX content was determined later as described previously (Nordholm, 1991).

Plasma concentrations of NBQX ranged between 17.7–27.3 μg ml^{-1} 7–9 min after the end of a 10 mg kg^{-1} infusion ($n = 4$) and $t_{1/2}$ was 1.3 ± 0.5 h ($n = 5$). This half-life allowed the use of two cumulative doses of NBQX in the cat.

2.5. Experimental protocols

All animals were recorded unrestrained throughout the experiment. For each control session, we retained the lowest ventilation value (V_E) that occurred during quiet waking or sleep in adult cats (determined according to behavioral and electroencephalographic criteria), and periods of immobility in mice and neonates. Control records lasted at least 15 min in adult animals and kittens, and 2 min in neonate mice. Each adult cat was given dizocilpine alone, NBQX alone, and dizocilpine followed by NBQX. Kittens and mice were given either NBQX alone, or NBQX followed or preceded by dizocilpine. Following a single or the second of two injections, data were averaged every 5 min, for 30 min with mice and up to 1 h with cats. We present in Section 3 the maximal effects produced on minute volume, which usually occurred 10–20 min after the injection. Injections of vehicle alone did not significantly affect respiratory activity in adult animals and kittens. However, in neonate mice, vehicle injection significantly decreased minute ventilation at +5 min (−26%, $n = 7$) and had no effect thereafter. Thus, after drug injections, we discarded the data collected between 0–5 min. Pilot studies showed the minimal doses of NBQX and combinations with dizocilpine that had a significant effect for each species/age and maximal doses producing no effect. It was also verified that changing the route of administration (s.c. or i.p.) in 2 adult and 4 neonate mice did not notably affect the effect of NBQX on ventilation. All animals except adult cats were used only once.

The effects of anesthesia on the ventilatory response to NBQX were determined in instrumented cats, which were given NBQX in the awake state and in the anesthetized state. Each experiment was followed by at least one-week's recovery period.

The results were expressed as means ± S.E.M. and comparisons between two sets of data were performed by repeated measures analysis of variance (ANOVA) and by paired Student's *t*-test. Averaged values for different strains were compared using unpaired *t*-tests.

2.6. Drugs

The drugs used were NBQX (Novo Nordisk, Måløv, Denmark), dizocilpine (Research Biochemicals International), pentobarbital (Sanofi, Libourne, France), halothane (Belamont, Paris, France).

3. Results

3.1. Ventilation in unanesthetized neonate and adult cats and mice

In accordance with previous results (review in Mortola and Noworaj, 1985), we found that breathing patterns undergo important changes during development. Breathing frequency (F) in neonate cats was about twice as high as in adult cats, and tidal volume per unit of body weight (V_T) was also greater (Table 1). Consequently, minute volume (V_E) in kittens was three times the value found in adults. Breathing patterns in developing mice evolve differently from those in cats, because of their small size, fast breathing frequency and limitations imposed by respiratory mechanics (Mortola and Noworaj, 1985). As opposed to cats, adult mice breathed faster than neonates, and V_E did not significantly change with growth. In both species, the relative fraction of the respiratory cycle occupied by the inspiratory phase (T_i/T_{tot}) increased during development.

3.2. Effects of NBQX administered alone

3.2.1. Adult mice

Adult mice were recorded as controls during periods of immobility which had lasted for at least 10 s when data

Table 1
Baseline respiratory variables (mean ± S.D.) in neonate and adult mice and cats

	Neonate cats ($n = 15$)	Adult cats ($n = 10$)	Neonate mice ($n = 26$)	Adult mice ($n = 16$)
Age	0–2 days		0–2 days	
Body weight (g)	122 ± 10	2730 ± 500	1.9 ± 0.3	25.3 ± 4.2
T_i (s)	0.31 ± 0.08	0.85 ± 0.15[b]	0.138 ± 0.037	0.099 ± 0.021[b]
T_e (s)	0.59 ± 0.15	1.11 ± 0.39[b]	0.314 ± 0.102	0.178 ± 0.050[b]
T_i/T_{tot} (%)	34.8 ± 4.2	44.4 ± 5.8[b]	31.0 ± 4.5	36.2 ± 4.9[b]
F (breaths min^{-1})	71 ± 19	32 ± 8[b]	143 ± 38	231 ± 64[b]
V_T (ml kg^{-1})	15.3 ± 3.6	9.5 ± 1.5[b]	9.7 ± 2.3	8.1 ± 1.9[a]
V_E (ml min^{-1} kg^{-1})	1050 ± 188	302 ± 92[b]	1.42 ± 0.60	1.84 ± 0.79

T_i: duration of inspiration.
T_e: duration of expiration.
T_i/T_{tot}: inspiratory fraction of respiratory cycle.
F: breathing frequency.
V_T: tidal volume.
V_E: minute volume.
n: number of animals.
[a] $P < 0.05$.
[b] $P < 0.001$ vs. neonates.

collection started. A large dose of NBQX (200 mg kg^{-1} i.p.) induced hypoventilation and hypothermia. V_E and body temperature decreased by 49 ± 6% and 3.9 ± 0.7°C, respectively ($n = 3$, both $P < 0.05$). Thus, in 5 other mice, external heating was used between each data collection to maintain body temperature at 37–38°C. All animals became ataxic and sedated within a few minutes, but still responded to noxious stimuli by withdrawal movements. Despite this severe behavioral toxicity, ventilation was not depressed on the average throughout a 30-min record following the injection of NBQX (Fig. 1). F, V_T and V_E did not change significantly.

3.2.2. Neonate mice

Newborn mice responded to small doses of NBQX (s.c.) by depressed ventilation. A dose of 2 mg kg^{-1} decreased body movements and significantly decreased F, and 5 mg kg^{-1} decreased both F and V_E. The decrease in V_E was entirely due to decreased F (Fig. 1). T_e increased much more than T_i, which reduced the T_i/T_{tot} ratio. A large dose of NBQX (200 mg kg^{-1}) decreased V_e by 77% (Fig. 1). As with the smaller doses, this effect was entirely due to a decreased F, with no significant change in V_T. Since the animals were kept warm between recordings, the decreased V_E was not accompanied by a significant decrease in body temperature. Despite the decreased V_E, all animals survived the recording session.

3.2.3. Adult cats

In adult cats, a slow infusion of NBQX (20 mg kg^{-1} over 33 min) did not significantly depress V_E (Fig. 2). The only significant change in respiratory parameters at the end of the infusion period was a slight (25%) decrease in V_T. At that time, the animals were unconscious and had mostly low-voltage electroencephalographic patterns (see Fig. 3A).

3.2.4. Neonate cats

In kittens, NBQX (20 mg kg^{-1}, $n = 8$) did not affect breathing patterns, but most animals remained active (Fig. 2). Thus, we increased the dose to 40 mg kg^{-1} (20 mg kg^{-1} injected 17 min after the first dose, $n = 7$), which sedated the animals and decreased V_E by 33% (Figs. 2 and 5C). This reduced ventilation resulted from a decreased F, which was incompletely compensated for by an increased V_T.

3.3. Combined effects of NBQX and anesthetics

We had shown previously that anesthesia augments the respiratory effects of dizocilpine (Foutz et al., 1988b). Here we infused NBQX (0.6 mg kg^{-1} min^{-1}, final dose 20 mg kg^{-1}) to adult cats during the conscious state, and, after recovery, during anesthesia with sodium pentobarbital (35 mg kg^{-1} i.v., $n = 4$) or halothane (2% in oxygen, $n = 3$). Following a baseline record in the anesthetized state, NBQX infusion was initiated at the same rate as in the unanesthetized state, but because there was a profound respiratory depression, the perfusion was stopped when the dose reached 10 mg kg^{-1} (one animal given halothane died at 5 mg kg^{-1}). V_E reached a minimal value of 50.7 ± 3.8% of baseline within 5 min after the end of infusion (vs. 87.2 ± 2.5% in the awake state, $P < 0.001$), then slowly recovered. Fig. 3 shows successive experiments with the same animal, where NBQX was infused in the unanesthetized state, during anesthesia with pentobarbital, and during anesthesia with halothane. One-half the dose of NBQX which was ineffective in the conscious state profoundly depressed ventilation in the anesthetized state.

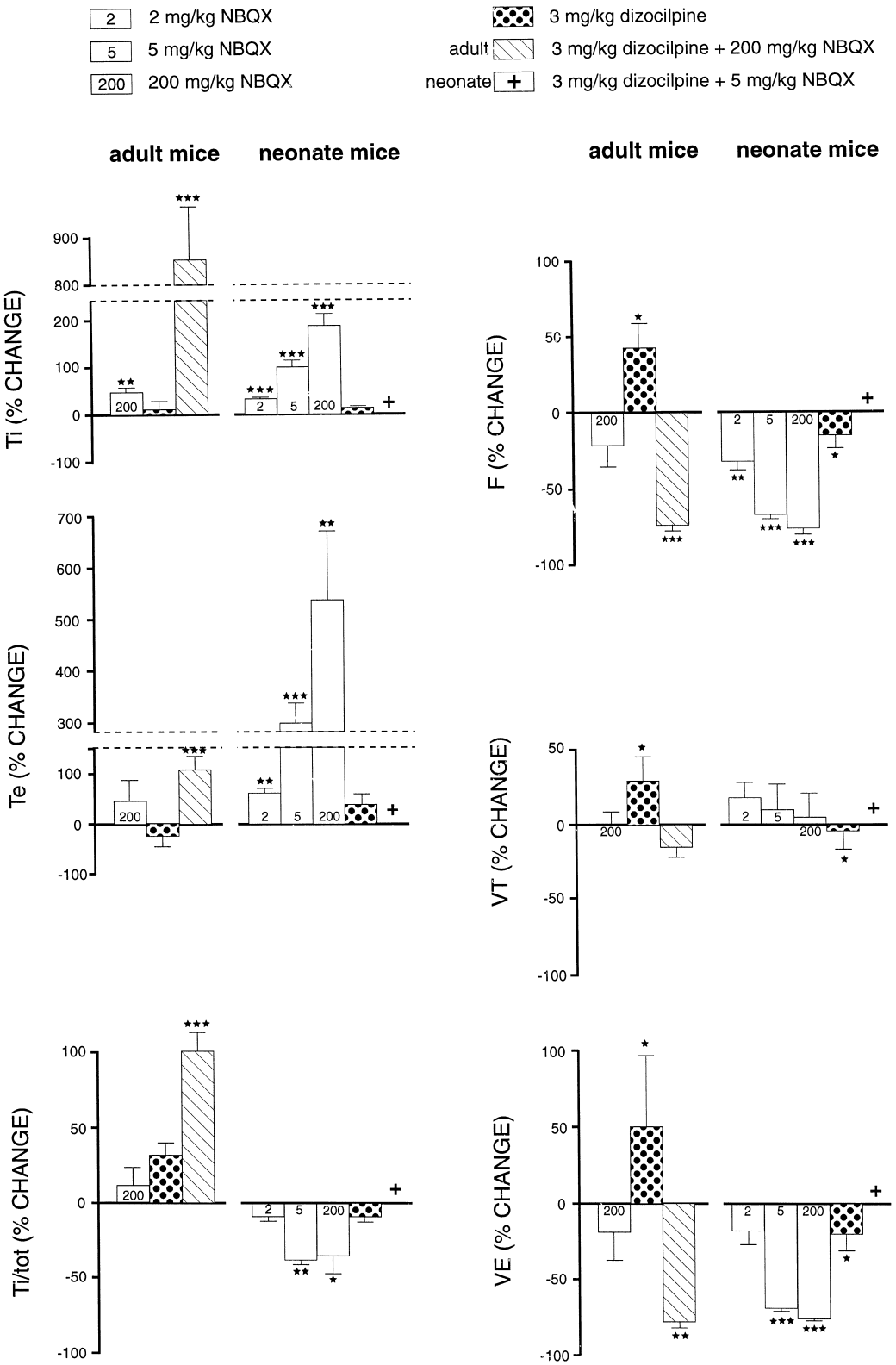

Fig. 1. Ventilatory effects of NBQX and dizocilpine, given separately and in combination to adult and to 0–2-day-old neonate mice. Values are expressed as maximal percentage changes from pre-drug control records. Doses of NBQX were 200 mg kg^{-1} ($n = 5$) in adults and 2 mg kg^{-1} ($n = 7$), 5 mg kg^{-1} ($n = 11$) and 200 mg kg^{-1} ($n = 8$) in neonates. The dose of dizocilpine was 3 mg kg^{-1} in adults ($n = 11$) and neonates ($n = 11$). This dose of dizocilpine was combined with NBQX, 200 mg kg^{-1} in adults ($n = 11$) and 5 mg kg^{-1} in neonates ($n = 11$). Abbreviations as in Table 1. $* P < 0.05$; $** P < 0.01$; $*** P < 0.001$. Black cross: lethal apnea.

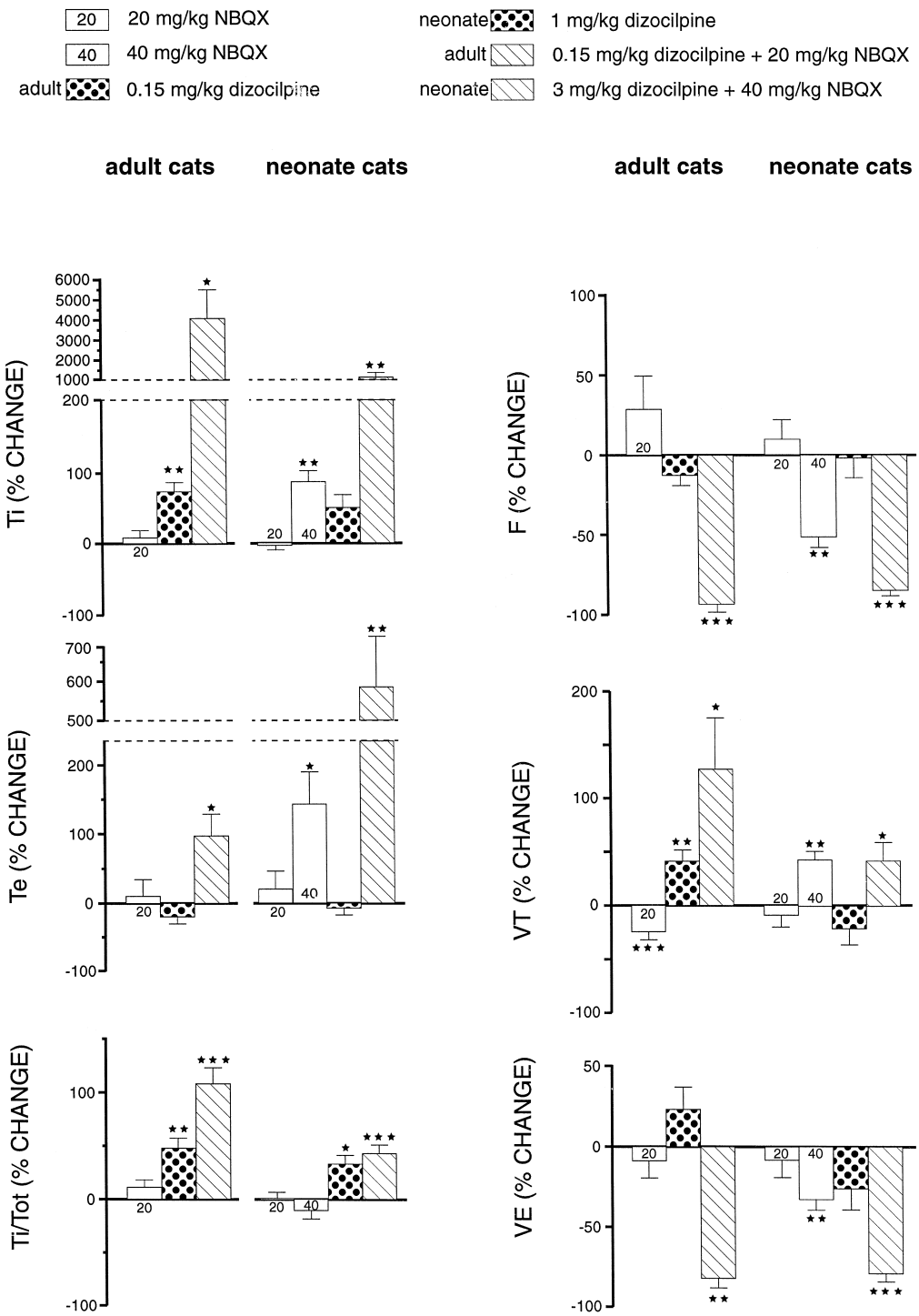

Fig. 2. Ventilatory effects of NBQX and dizocilpine, given separately and in combination to adult cats and to 0 to 2-day-old kittens. Values are expressed as maximal percentage changes from pre-drug control records. Doses of NBQX were 20 mg kg^{-1} ($n = 10$) in adults, 20 mg kg^{-1} ($n = 8$) and 40 mg kg^{-1} ($n = 7$) in neonates. The dose of dizocilpine was 0.15 mg kg^{-1} in adults ($n = 6$) and 1 mg kg^{-1} in neonates ($n = 5$). Dizocilpine was combined with NBQX, 20 mg kg^{-1} in adults ($n = 6$) and 40 mg kg^{-1} in neonates ($n = 8$). Abbreviations as in Table 1. $*\ P < 0.05$; $**\ P < 0.01$; $***\ P < 0.001$.

3.4. Effects of dizocilpine administered alone

3.4.1. Adult and neonate mice

In adult mice dizocilpine (3 mg kg^{-1}, $n = 11$) had no anesthetic effect and stimulated ventilation (Figs. 1 and 4). V_E increased by 50% 10–15 min after the injection. This was due to significant increases in both F and V_T. In neonate mice, however, dizocilpine ($n = 11$) depressed ventilation (Figs. 1 and 5A). This resulted from small but significant decreases in both F and V_T. Thus, dizocilpine has opposite respiratory effects in adult and in neonate mice.

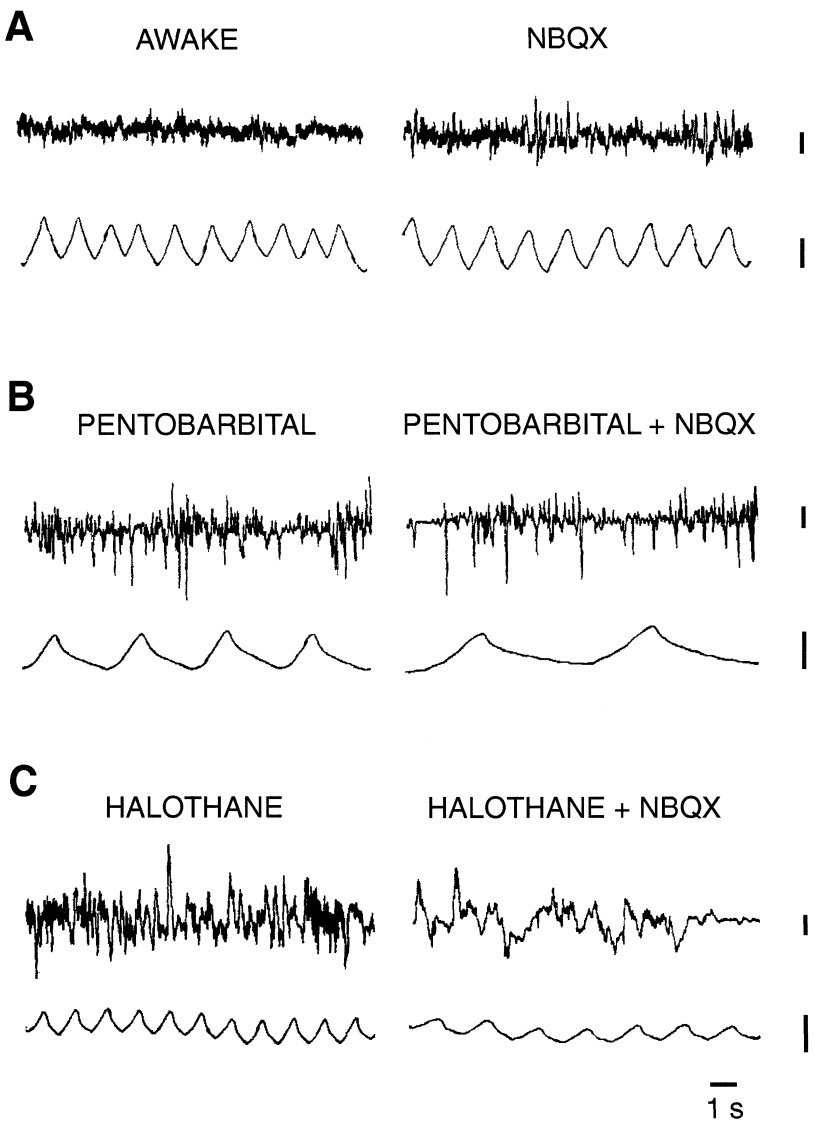

Fig. 3. Effects of successive NBQX infusions at least one week apart in a chronically instrumented cat. Upper traces: electrocorticogram; lower traces: plethysmographic record. The dose of NBQX was 20 mg kg^{-1} in the unanesthetized state (A), 10 mg kg^{-1} during anesthesia with pentobarbital (B) and halothane (C). Calibration bars: 50 μV and 20 ml. Inspiration up.

3.4.2. Adult and neonate cats

In adult cats, dizocilpine (0.15 mg kg^{-1}, $n = 6$) had a sedative effect and prolonged T_i but not T_e, thus increasing the T_i/T_{tot} ratio (Fig. 2). V_T increased but not V_E. We had previously shown that higher doses (0.3–1 mg kg^{-1}) slightly depress ventilation (Foutz et al., 1988b). In neonate cats, dizocilpine (1 mg kg^{-1}) affected, as in adults, the timing of respiratory phases and the T_i/T_{tot} ratio, but did not significantly depress V_E (Fig. 2).

3.5. Combined effects of NBQX and dizocilpine

3.5.1. Adult mice

In adult mice NBQX (200 mg kg^{-1} injected immediately after the recording of dizocilpine effects, $n = 11$), provoked a profound change in the breathing pattern, which became apneustic, with maximal effects attained 15–30 min after NBQX (Figs. 2 and 4). Each inspiration was followed by a pause, which prolonged the average inspiratory time by a factor of 9 (from 0.15 ± 0.016 s with dizocilpine to 0.846 ± 0.120 s, range 0.35–1.56 s). Consequently, F and V_E decreased strongly. The decrease in V_E was almost entirely due to the decreased F because V_T was not significantly affected. At this stage of the experiment, the animals were breathing 100% 0_2, and none died during the observation period.

3.5.2. Neonate mice

In neonate mice aged 0–2 days, associating dizocilpine with NBQX never produced apneusis but rapidly induced apnea. Small doses of NBQX (2 mg kg^{-1}, $n = 2$, or 5 mg kg^{-1}, Fig. 1), injected 15–20 min after dizocilpine (3 mg

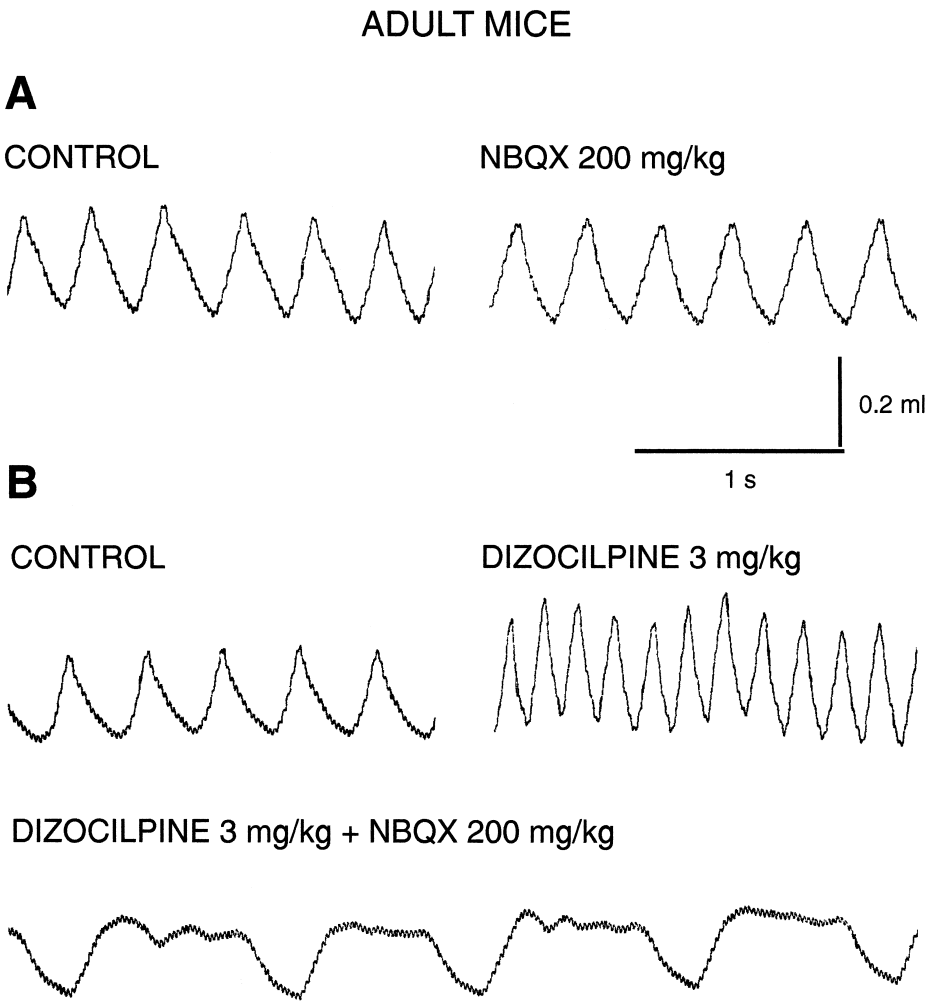

Fig. 4. Separate and combined effects of NBQX and dizocilpine on the breathing pattern in adult mice. (A) A large dose of NBQX did not affect the breathing pattern. (B) In another animal, dizocilpine increased ventilation, but the addition of NBQX produced an apneustic breathing pattern. Inspiration: upward deflection.

kg^{-1}), further decreased V_T and V_E, and all animals died within 2–18 min. This final apnea often occurred rapidly over a few respiratory cycles (Fig. 5A). T_e increased greatly, and V_T decreased until complete respiratory arrest. Between 30–60 s before respiratory arrest, V_T and V_E had decreased by 61% ± 3% and 69% ± 5%, respectively.

Pups aged 9–10 days ($n = 5$) were given the dosage combination (dizocilpine 3 mg kg^{-1} and NBQX 5 mg kg^{-1}) that killed newborn pups. All survived through 30 min recordings but did not show apneusis. In other pups, the dose of NBQX was increased to 25 mg kg^{-1}. Given alone ($n = 2$), it depressed ventilation, but did not cause apneusis, while dizocilpine alone ($n = 6$) significantly reduced V_T and V_E (by about one-third), without affecting timing mechanisms (Fig. 5B). Addition of NBQX (25 mg kg^{-1}, $n = 11$) to dizocilpine further decreased V_E, and slowed breathing greatly by increasing both T_i (by 500%) and T_e (by 1000%). Most remarkably, an apneustic pattern with inspiratory 'holds' sometimes exceeding 0.5 s was observed in 6/11 animals (Fig. 5B), and small inspiratory pauses occurred in two others. Ten of eleven animals survived.

In younger mouse pups aged 7 days ($n = 2$), the combined effect of dizocilpine and NBQX (25 mg kg^{-1}) produced, interspersed among normal breaths, short inspiratory 'holds' not exceeding 150 ms.

3.5.3. Adult cats

In adult cats, dizocilpine (0.15 mg kg^{-1}) administered 7 min after the end of a slow NBQX infusion (20 mg kg^{-1}), produced an extremely pronounced apneusis. T_i increased 40-fold while T_e merely doubled (Fig. 2). F thus decreased by 90%, which could not be compensated for by an increase of V_T, so V_E decreased by 80%.

3.5.4. Neonate cats

In neonate cats, two protocols were used so that the effects of NBQX and dizocilpine could first be tested separately. In protocol 1 ($n = 5$), NBQX was administered after dizocilpine (1 mg kg^{-1}), in two doses (20 mg kg^{-1})

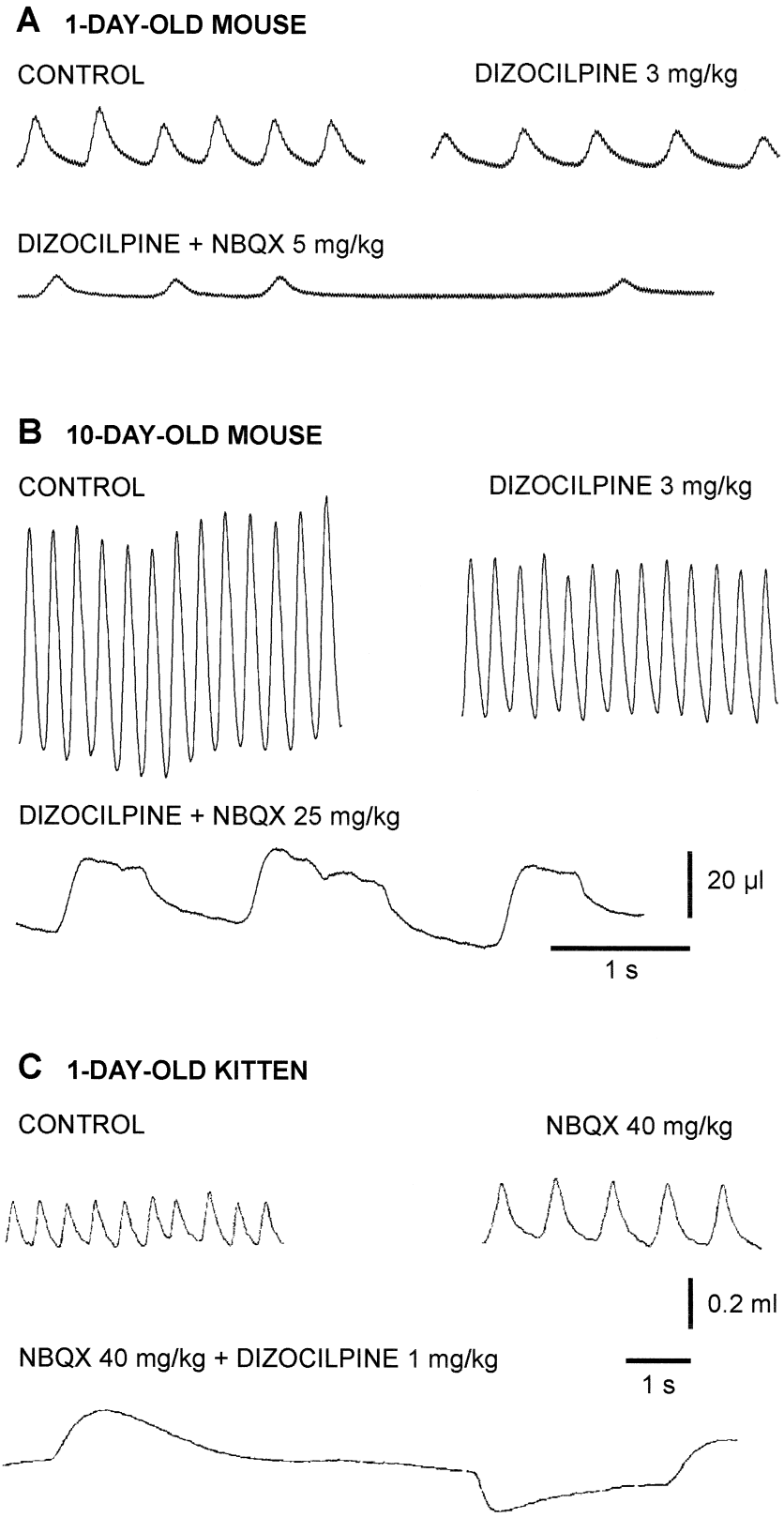

Fig. 5. Effects of dizocilpine, NBQX, and both compounds in 1-day-old mice (A), 10-day-old mice (B) and a 1-day-old kitten (C). Inspiration up. The drug combination produced apnea in the newborn mouse and apneusis in the older mouse and in the kitten.

separated by 22–29 min. Apneusis developed in 4/5 animals. In protocol 2 ($n = 5$), the drugs were administered in reverse order: two doses of NBQX, 16 min apart, followed by dizocilpine 30 min after the second dose of NBQX (Fig. 5C). Apneusis developed in 3/5 animals. The two others (one of which presented with apneas during the control recordings), rapidly developed a terminal apnea and could not be included in the database. The results of the two administration protocols were pooled in Fig. 2. The association of both drugs increased T_i 12-fold (to 3.71 ± 0.76 s) and T_e 7-fold (to 3.99 ± 0.91 s). Consequently, as in adults, F decreased by 85%, and V_E by 80% despite a significant increase in V_T. The association of dizocilpine and NBQX thus affected most respiratory parameters in a remarkably similar way in newborn and in adult cats, but the expiratory phase was more prolonged in neonates than in adults.

4. Discussion

The present results showed that doses of glutamate receptor antagonists higher than required for neuroprotection, are relatively innocuous for respiratory function in adult animals if they are administered in the awake state, and if they block selectively one receptor subtype. Blockade of both non-NMDA and NMDA receptors always disrupts ventilation in neonates and adults. Furthermore, non-NMDA receptor antagonism depresses respiratory activity in neonate mice but not in adult mice, whereas receptor specialization in respiratory timing mechanisms is present at birth in the cat.

4.1. Dizocilpine has minor effects on ventilation

Dizocilpine in a dosage of 1 mg kg^{-1} completely blocks the excitatory effect of NMDA applied by iontophoresis onto bulbar respiratory neurons in the cat (Foutz et al., 1988a) and in the rat (Connelly et al., 1992). The present and previous results (Foutz et al., 1988b) indicate that large doses of dizocilpine (≥ 1 mg kg^{-1}), which presumably completely block NMDA receptors involved in respiratory function, do not have or a slightly depressive effect in cats and neonate mice. However, ventilation was increased in adult mice. Since, unlike cats, adult mice were not anesthetized with dizocilpine, this paradoxical increase was attributed to a stimulating action of dizocilpine on forebrain structures controlling respiration (Cassus-Soulanis et al., 1995).

4.2. NBQX has minor effects in adults and species-dependent effects in neonates

The highest dose of NBQX (200 mg kg^{-1}) administered to mice in this study was greater than doses reported to be anticonvulsant in mice (Chapman et al., 1991; Swedberg et al., 1995) and to provide neuroprotection in a rodent model of ischemia (Gill et al., 1992). This large dose did not affect respiratory activity in adult mice despite severe behavioral impairment. However, doses as low as 5 mg kg^{-1} profoundly depressed respiration in neonate mice. Thus, in neonate rodents, respiration depends critically on activation of non-NMDA receptors, in keeping with results obtained in vitro (Liu et al., 1990; Funk et al., 1993). This pronounced age-related effect of NBQX was not observed in the cat. Respiration was unaffected in adult cats by 20 mg kg^{-1} of NBQX, although a dose of 12 mg kg^{-1} infused at the same rate blocked most of the excitatory effect of a non-NMDA receptor agonist applied by iontophoresis onto respiratory neurons (Pierrefiche et al., 1994). Only a still higher dose (40 mg kg^{-1}) depressed V_E in kittens. Thus, respiratory function may be more mature in the neonate cat than in the neonate mouse. In neonates of both species, NBQX always acted by decreasing F but not V_T, and thus affected primarily the rhythm generator.

4.3. Respiratory depressant effect of NBQX during anesthesia

The depression of ventilation produced during anesthesia by one half the dose of NBQX, which was ineffective when given to the same adult cats in the awake state, demonstrates a synergistic action of NBQX with anesthetics, and is consistent with previous results obtained in rodents (McFarlane et al., 1992; Dall et al., 1993). We had shown previously that anesthetics also increase the respiratory effects of NMDA receptor blockade (Foutz et al., 1988b). Interactive effects with the anesthetic might also explain the severe respiratory impairment following low doses of NBQX and dizocilpine in chloralose-anesthetized cats (McManigle et al., 1994). An obvious consequence of this synergistic action is that the respiratory effects of glutamate receptor antagonists can only be compared meaningfully under standardized experimental conditions (anesthetized or not).

4.4. Respiratory toxicity of combined blockade of NMDA and non-NMDA receptors

The combined blockade of both receptor types was toxic in adults and neonates alike. An entirely different pattern of breathing, apneusis, developed in cats, kittens and adult mice, and a lethal apnea occurred in neonate mice. The apneustic respiratory pattern depressed ventilation through an extreme slowing of breathing frequency, but the amplitude of respiratory movements was unaffected in adult mice and actually increased in cats. Thus, glutamate antagonism affected primarily respiratory timing mechanisms, with no apparent impairment of the transmission of respiratory drive except in neonate mice. This lack of effect on respiratory amplitude, and the requirement of a combined receptor blockade for producing apneusis, are

surprising because glutamate ensures fast neurotransmission in the respiratory network, as in other brain networks, by activating both NMDA and non-NMDA receptors, which are believed to act cooperatively (Bonham, 1995; Denavit-Saubié and Foutz, 1996).

4.4.1. Respiratory timing mechanisms

Apneusis can be explained by the fact that, in mammals, inspiratory activity is terminated independently by two distinct sources of synaptic inputs to the bulbar respiratory Central Pattern Generator. One input originates from the slowly adapting pulmonary stretch receptors via the vagus nerves, the other from neurons in the 'pneumotaxic center' in the upper pons, and each input activates a distinct glutamate receptor subtype (review in Bianchi et al., 1995). Thus, after vagotomy, apneusis is produced by NMDA receptor antagonists microinjected in the pneumotaxic centre (Fung et al., 1994) or in the Nucleus Tractus Solitarius (Berger et al., 1995), or administered systemically (review in Denavit-Saubié and Foutz, 1996), but non-NMDA receptor antagonists fail to induce apneusis (Pierrefiche et al., 1994). This shows that the pneumotaxic mechanism acts through activation of NMDA receptors without critical involvement of non-NMDA receptors. In contrast, afferent pathways used by pulmonary stretch receptors to terminate inspiration involve essentially non-NMDA receptors (Bonham et al., 1993; Pierrefiche et al., 1994). Together, these data indicate that NMDA receptor antagonists block synapses activated by pneumotaxic afferents, whereas non-NMDA receptor antagonists block the vagal afferent pathway mediating the Hering–Breuer reflex. The present results suggest that receptor specialization in both vagal and pneumotaxic afferent pathways to the Central Pattern Generator is completed at birth in the cat. However, we could not observe a pharmacologically induced apneusis before day 7 in the mouse. This result was unexpected because lesioning and stimulating experiments have demonstrated that both the pneumotaxic center (Fung and St John, 1995) and vagal afferent pathways that terminate inspiration (Paton and Richter, 1995) are functional from the first day of life in rats and mice, respectively. Thus, the failure to induce apneusis pharmacologically in the newborn mouse might have been due to the toxic effects of the drug combination, small doses of NBQX associated with dizocilpine, which rapidly killed the animals in apnea, whereas larger doses were required to produce apneusis in older mice and in kittens. Glutamate antagonism also increased the length of the expiratory phase, probably by acting directly on the Central Pattern Generator (Funk et al., 1993). This effect was greater in neonate than in adult cats.

4.4.2. Respiratory motor output

The increased tidal volume following generalized blockade of ionotropic glutamate receptors in the cat is paradoxical because the transmission of respiratory drive within the brainstem respiratory network and to phrenic motoneurons involves an excitatory amino acid that activates both non-NMDA and NMDA receptors in adults (Pierrefiche et al., 1991, 1992, 1994; Böhmer et al., 1991; Abrahams et al., 1993). However, when the respiratory motor output is completely suppressed by glutamate receptors antagonism in animals maintained with constant blood gases by artificial ventilation, the output can be re-established by increasing P_aCO_2 (Pierrefiche et al., 1994). This led us to hypothesize (Foutz et al., 1994) that, in spontaneously breathing animals, activation of chemosensitive mechanisms by carbon dioxide buildup induces the recruitment of more motoneurons. The present results suggest that neonate cats, but not neonate mice, have a sufficiently mature 'synaptic vital capacity' to overcome the effects of a synaptic blockade sufficient to disrupt the phase transition mechanism.

4.5. Conclusion

In a review of early work on the use of AMPA/kainate receptor antagonists in models of cerebral ischemia, Gill (1994) concluded that combinations of AMPA and NMDA receptor antagonists will not necessarily result in additive neuroprotective effects. However, more recent work suggests that very low doses of NMDA receptor antagonists may indeed act synergistically with AMPA/kainate antagonism for neuroprotection (Löscher et al., 1993; Lippert et al., 1994). Our results show that these two classes of drugs unfortunately also act synergistically to depress respiration in both adults and neonates. The severity of this depression appears to be species-dependent and age-dependent. It might thus be indicated to evaluate the effects of any proposed cocktail of glutamate receptor antagonists on respiratory function.

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