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BRIEF COMMUNICATION

Yohimbine Reduces Neuropathology Induced by Ketamine/Xylazine Anesthesia

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KILANDER, K. AND H. WILLIAMS. *Yohimbine reduces neuropathology induced by ketamine/xylazine anesthesia.* PHYSIOL BEHAV **51**(3) 657–659, 1992.—Ketamine, an NMDA receptor antagonist, is commonly used in combination with xylazine, an α_2-adrenergic receptor agonist, to induce surgical anesthesia in birds and other vertebrates. Problems associated with this anesthetic combination include impaired thermoregulation, compounded by the inability to feed while anesthesia lasts (approximately 6 h after a single dose), and the ketamine-induced vacuolization of large cortical neurons. In the zebra finch, yohimbine (an α2-adrenergic receptor agonist) counters the effects of ketamine/xylazine anesthesia, speeding recovery after the surgical procedure has been completed. In addition, administration of yohimbine reduces the formation of vacuoles in large cortical neurons and in neuropil. Yohimbine administration should be considered following all procedures involving ketamine/xylazine anesthesia.

Ketamine Xylazine Yohimbine Neuropathology Zebra finch Electron microscopy Cortex Vacuolization

KETAMINE, an NMDA-receptor antagonist related to phencyclidine (8), is a common and effective anesthetic most often used in combination with xylazine, an α_2-adrenergic receptor agonist, for recovery surgery on mammals (2,4,5,6,9) and birds (1). Ketamine/xylazine administered by intramuscular injection provides long-lasting surgical anesthesia and is convenient for use in many procedures where the apparatus used for administering inhaled anesthetics is inconvenient. However, there are disadvantages to ketamine/xylazine as an anesthetic. Some procedures are relatively short compared to the duration of anesthesia, leading to unnecessary thermal and metabolic stress on the animal. In addition, ketamine anesthesia results in the formation of large vacuoles in the cortical neurons of rats, and it has been suggested that behavioral changes following drug usage may be related to the formation of the vacuoles (11). Many studies in behavioral neuroscience use ketamine/xylazine anesthesia, and such effects complicate the interpretation of post-surgical behavior.

The effect of yohimbine, an α_2-adrenergic receptor agonist, in countering ketamine/xylazine anesthesia (presumably because of its relationship to xylazine) has been documented in several mammals, including chinchillas (2), rats (4), cats (5), rabbits (6), and red deer (9), although it is not effective in Rhesus monkeys (7). Complications due to long periods of unconsciousness were reduced, since the animals became self-sufficient more quickly. Yohimbine, like ketamine/xylazine anesthesia, is injected intramuscularly, and is readily available, as well as convenient. In this study, we examine the effects of yohimbine upon recovery from surgical anesthesia and the formation of vacuoles in the neurons of ketamine/xylazine-anesthetized zebra finches.

METHOD

Subjects were female zebra finches (n = 8) weighing 10 g, bred on site and maintained with food, water, and grit ad lib on a 14h:10h light:dark photoperiod. Two groups (A and B) of three birds were chosen at random and used in testing recovery from anesthesia. Both groups were administered ketamine (Ketalar, Parke-Davis; 25 mg/kg) and xylazine (AnaSed, Lloyd Laboratories; 50 mg/kg). Ten minutes after surgical anesthesia (defined as lack of response to a toe pinch) was attained, group A received an injection of yohimbine (Sigma; 1.25 mg/kg in distilled water). The dosage of yohimbine used was initially determined by extrapolating linearly from the dosage level used in the chinchilla (1), and corresponds to 1/40 (by weight) of the amount of xylazine injected. The initial dosage (25 mg/kg) was fatal, and was diluted 20× to achieve a satisfactory effect.

After all injections were given, the birds were placed on a circulating water heat pad, and under a 25-W red bulb that served as a heat lamp. They were allowed to recover with no further interference, and the level of anesthesia was monitored continually until all had recovered. The time to induction of surgical

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TABLE 1

YOHIMBINE COUNTERS KETAMINE/XYLAZINE ANESTHESIA

	Onset of Surgical Anesthesia	Duration of Surgical Anesthesia	Time to Head Lift	Time to Recovery
Yohimbine	5.2 ± 3.0	17.9 ± 0.9	36.3 ± 9.4	81.4 ± 21.6
Control	3.6 ± 0.9	54.2 ± 15.4	70.4 ± 22.6	248.2 ± 58.1
$t(10)$	1.28	5.58	3.42	6.59
	$p > 0.2$	$p < 0.001$	$p < 0.01$	$p < 0.001$

Mean times in minutes ± SD. Values within each of the two groups are pooled; there were no significant effects of treatment order. Differences between the yohimbine and control groups were assessed with a t-test.

anesthesia, duration of surgical anesthesia, time to first head lift, and time to full recovery, including flight were recorded. Two weeks later, the procedure was repeated, with group B receiving yohimbine injections and group A serving as controls.

The remaining two birds were injected with the ketamine/xylazine combination at the standard dosage level. One bird then received yohimbine at the dosage described above. Both birds were perfused (4% paraformaldehyde in 0.1 M pH 7.4 phosphate buffer) six hours after anesthesia was induced, and the HVC, a portion of the neostriatum known to be related to song learning and production (10) was removed, embedded in Epon, sectioned at 80 nm, and examined and photographed with a Philips CM-10 transmission electron microscope.

RESULTS

General Observations

In the control birds, uncoordinated movements (wing flapping, kicking) appeared earlier than alertness (defined by such characteristics as open eyes and erect head position). Controls often moved into awkward postures at the corners of the cage during bouts of thrashing movements. In contrast, yohimbine-injected birds appeared to become alert before attempting to move, first raising their heads, opening their eyes, and moving their necks in a way that allowed them to examine their surroundings.

Duration of Anesthesia

Yohimbine treatment dramatically decreased the recovery time after anesthesia. The mean duration of surgical anesthesia (which yohimbine treatment reduced by 67%), the time to head lift (−49%), and the time to full recovery (−67%) were all significantly less in birds that received yohimbine (Table 1). As would be expected, the mean time for induction of surgical anesthesia did not differ significantly between birds in the control and yohimbine groups.

Vacuole Formation

Cortical neurons with extensive vacuoles were abundant in the bird that received only ketamine/xylazine anesthesia, but were not apparent in the bird that also received yohimbine [Fig. 1(A,B)]. In addition, regions of extensive vacuolization in the neuropil as well as large vacuoles in the somata of neurons were common in material from the control bird, and relatively rare in the bird that received yohimbine [Fig. 1(C,D)].

DISCUSSION

Small doses of yohimbine dramatically reduced the duration of ketamine/xylazine anesthesia in zebra finches, the first time this has been demonstrated in a bird. Yohimbine also smoothed the behavioral transition to recovery, eliminating the thrashing movements that often characterize emergence from ketamine anesthesia.

Yohimbine treatment decreased the ketamine-induced formation of vacuoles in the forebrain. The effects of these vacuoles on neural function is unknown, but vacuole formation is induced by the PCP family of psychoactive drugs (11). If the appearance of vacuoles is related to the behavioral abnormalities that are

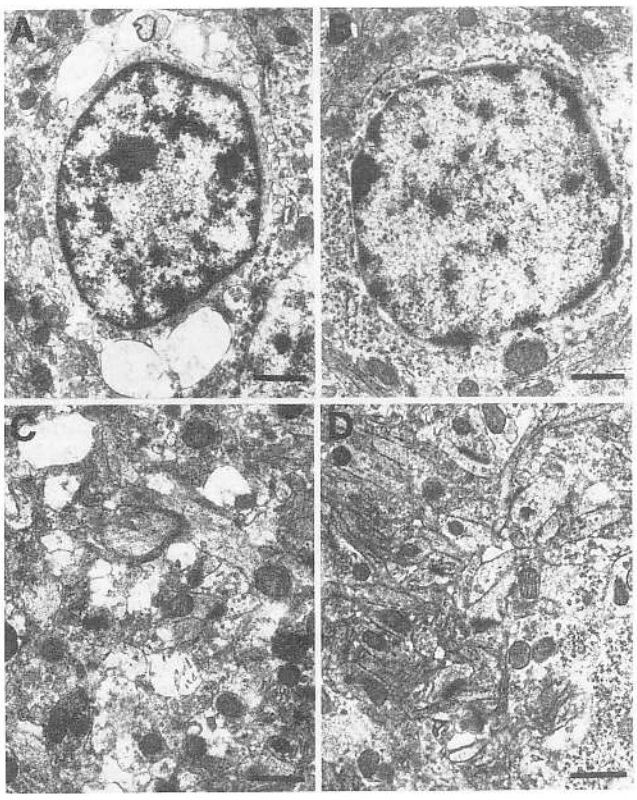

FIG. 1. Vacuoles are reduced after yohimbine treatment. Electron micrographs of the HVC region of the neocortex, fixed 6 h after the administration of ketamine/xylazine anesthesia. (A) Neuron with large vacuoles in the cytoplasm, containing structures that appear to be portions of mitochondria. (B) An example of a neuron from the same region after yohimbine treatment; no vacuoles appear in the cytoplasm. (C) Neuropil from the HVC region, with many vacuoles present in presynaptic and postsynaptic endings. (D) Fewer vacuoles can be found in the neuropil of the neocortex in the HVC region after yohimbine treatment. In all cases, scale bars represent 1 μ.

also associated with PCP-related drugs, yohimbine may serve to ameliorate such behavioral effects.

As yohimbine is related in function to xylazine, and not ketamine, the reduction in vacuoles is presumably not due to a direct effect. Ketamine noncompetitively blocks NMDA channels, and the duration of the block is reduced by depolarization related to neural activity (2). Yohimbine, in countering the effects of xylazine, presumably brings about a general increase in neural activity that could in turn act heterosynaptically to depolarize neurons with NMDA receptors. Such an indirect mechanism could then counter ketamine's effects and so reduce vacuolization in cortical neurons.

The striking effects of yohimbine upon ketamine-induced anesthesia make it a strong candidate for routine use as part of the standard regimen of recovery surgery whenever ketamine/xylazine anesthesia is used. The presence of large vacuoles in neurons for 24–48 hours after ketamine administration suggests that behavioral evaluations during this period may be compromised; yohimbine allows for earlier behavioral assessments. From a humanitarian standpoint, suffering due to thermal and behavioral stress after recovery surgery using ketamine/xylazine anesthesia is relieved by the administration of yohimbine. For both of these reasons, yohimbine is recommended after ketamine/xylazine anesthesia.

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