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KYNURENIC ACID-INDUCED PROTECTION OF NEUROCHEMICAL AND BEHAVIOURAL DEFICITS PRODUCED BY QUINOLINIC ACID INJECTIONS INTO THE NUCLEUS BASALIS OF RATS

RICHARD J. BENINGER[1,*], KHEM JHAMANDAS[2], ROLAND J. BOEGMAN[2] and SHERIF R. EL-DEFRAWY[2]

Departments of [1]Psychology and [2]Pharmacology and Toxicology, Queen's University, Kingston, Ont. K7L 3N6 (Canada)

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Injection of the endogenous tryptophan metabolite, quinolinic acid (120 nmol in 1.0 μl) unilaterally into the basal forebrain of rats resulted in a significant ipsilateral decrease in cortical choline acetyltransferase activity, suggesting that cholinergic cells of the nucleus basalis magnocellularis (nbm) were damaged. Injected animals also showed a significant deficit in performance on an 8-arm radial maze, compared to sham operated controls, indicating an impairment of memory. Co-injection of another endogenous tryptophan metabolite, kynurenic acid (360 nmol in 1.0 μl) with quinolinic acid afforded an almost complete protection against the neurotoxic and memory-impairing effects of quinolinic acid alone. These findings support previous reports that kynurenic acid can protect against the neurotoxic effects of quinolinic acid and indicate for the first time that kynurenic acid can also protect against impairments of memory produced by injection of quinolinic acid into the nbm.

The nucleus basalis magnocellularis (nbm), located in the basal forebrain, contains cholinergic cell bodies that project to the cerebral cortex [2, 15, 25]. Recently it has been shown that the endogenous tryptophan metabolite, quinolinic acid [8], produces axon sparing lesions [30], and injections of this agent into nbm produce significant decreases in cortical cholinergic function [13, 14]. Furthermore, co-injection of another endogenous tryptophan metabolite, kynurenic acid, with quinolinic acid provides an almost total biochemical protection of the basal forebrain cells from the toxic effects of quinolinic acid. Thus cortical cholinergic markers remain unchanged [7, 18]. This latter observation has led to the suggestion that a change in the balance between these two compounds may be a factor in neurodegenerative diseases involving the loss of basal forebrain cholinergic cells [31].

In the past 3 years, a large number of studies has shown that memory for recent events is impaired in animals with lesions of the nbm. This has been shown in passive avoidance, T-maze alternation, matching to sample and radial maze tasks following electrolytic, kainate or ibotenic acid lesions [1, 4, 5, 9, 11, 12, 16, 19, 20, 22, 23, 26,

*Author for correspondence.

27, 29]. Fewer studies have reported the effects of quinolinic acid lesions on memory. We found that unilateral injections into the basal forebrain resulted in a significant unilateral loss of cortical choline acetyltransferase (ChAT) and an impairment in memory in a T-maze alternation task [6]. We now report for the first time that unilateral quinolinic acid lesions of the basal forebrain impair memory in an 8-arm radial maze task and, furthermore, that this memory impairment is prevented by co-injection of kynurenic acid with quinolinic acid.

Twenty experimentally naive male Sprague–Dawley rats, obtained from Charles River Canada, weighed 215–265 g at the beginning of the experiment and were maintained at approximately 85% of their free-feeding weights, adjusted for growth, by daily feeding with measured rations. Rats were housed individually in wire mesh cages in a climatically controlled (21 ± 1 °C) colony room kept on a 12 h light (06.00–18.00 h)/dark cycle with water continously available.

The maze, elevated 50 cm above the floor, consisted of an octagonal central platform (30 cm wide) surrounded by 8 equally spaced radial arms (65 cm long × 10 cm wide), each with a food well (1.5 cm diameter, 1.0 cm deep) located 1 cm from the distal end. All rats received 42 days of training on the radial maze prior to lesions. At approximately the same time each day, 5 days a week, each rat was placed on the central platform with a 45-mg food pellet (Bioserv) located in the food well of a randomly selected set of 4 of the 8 arms. The baiting pattern varied from rat to rat but was always the same from day to day for an individual rat. Animals remained on the maze until all 4 pellets were eaten, 14 choices were made or 10 min elapsed, whichever came first.

Surgery followed the final training day. Rats were randomly assigned to sham ($n = 7$), quinolinic acid ($n = 7$) or co-injection of quinolinic acid and kynurenic acid ($n = 6$) groups. Each rat was anesthetized with halothane (Halocarbon, Malton, Ont., Canada; 2% halothane – 98% oxygen) and positioned in a stereotaxic frame. With the incisor bar set at 3.3 mm below the interaural line, unilateral microinjections were aimed at the nbm with coordinates from bregma being 0.8 mm posterior, 2.6 mm lateral to the midline and 8.0 mm ventral to the surface of the skull. All rats received 1.0 μl infused over 2.5 min, and the cannula (0.35 mm o.d.) was left in place for 3.0 additional min to allow for diffusion. Sham rats received 0.9% saline. Quinolinic acid (Sigma) was injected in a dose of 120 nmol titrated to pH 7.4 with 1 N NaOH. Co-injections included 120 nmol of quinolinic acid and 360 nmol of kynurenic acid (Sigma) similarly adjusted to pH 7.4.

After a recovery period of at least 2 weeks behavioural testing began. Each rat received one session per day for 4 days using the same baiting pattern that was employed prior to surgery. Following the final test session all rats were killed by decapitation and their brains removed. A section of front-parietal cortex was dissected from each hemisphere and assayed for ChAT by the method of Fonnum [17]. Protein was measured according to the method of Lowry et al. [24].

Biochemical and behavioural results are shown in Table I. Sham operated rats showed no decrease in ChAT as expected. Quinolinic acid produced a 47.8% decrease in ChAT activity on the injected side compared to the intact side for the lesion group.

TABLE I

MEAN (±S.E.M.) LEVELS OF CORTICAL ChAT ON THE INJECTED AND UNINJECTED SIDE, PERCENT DECREASE ON THE INJECTED SIDE AND TOTAL CORRECT CHOICES IN THE RADIAL MAZE FOR 4 DAYS BEFORE AND AFTER SURGERY FOR 3 GROUPS

*Different from sham, ANOVA, $P<0.001$. +Different from pre-surgery, ANOVA, $P<0.05$.

	Sham (n = 7)	Quinolinic acid (n = 7)	Co-injected (n = 6)
ChAT (nmol acetylcholine formed/mg protein/h)			
Injected side	53.7 ± 1.9	28.0 ± 3.5	50.3 ± 1.4
Uninjected side	54.7 ± 2.8	53.6 ± 2.4	51.6 ± 1.0
Decrease (%)	0.6 ± 4.5	47.8 ± 6.0*	2.3 ± 3.1
Total correct (maximum 16)			
4 days pre-surgery	12.7 ± 0.6	13.0 ± 0.5	12.0 ± 0.7
4 days post-surgery	12.5 ± 0.5	10.4 ± 1.0+	12.3 ± 1.1

In contrast, quinolinic acid co-injected with kynurenic acid produced a 2.3% decrease in ChAT activity. Thus co-injection of kynurenic acid afforded an almost total protection against the toxic effects of quinolinic acid. The significance of these effects was confirmed with a one-way analysis of variance (ANOVA) of the percent decrease data. The overall group effect was significant, $F_{2,17}=31.38$, $P<0.001$, and planned comparisons revealed that the quinolinic acid group differed significantly from the sham, $F_{1,12}=39.42$, $P<0.001$, whereas the co-injected group did not, $F_{1,11}<1.0$, $P>0.05$.

To assess the effects of treatments on performance of the maze task, the total number of correct responses (entries into baited arms) on the first 4 trials of each session were summed for the 4 sessions prior to surgery and for the 4 sessions following surgery. Perfect performance would yield a total correct score of 16. As seen in Table I, the 3 groups showed little difference in mean total correct responses prior to surgery. However, after surgery the quinolinic acid group made fewer correct responses, whereas the co-injected and sham groups continued to perform at pre-surgery levels. A two-way mixed design ANOVA with independent groups and repeated measures on the pre/post-surgery variable revealed a significant interaction, $F_{2,17}=3.49$, $P=0.05$, suggesting that surgery affected the performance of the groups differently. Planned comparisons of pre-surgery and post-surgery total correct for each group revealed a significant decrease for the quinolinic acid group, $F_{1,6}=6.07$, $P<0.05$, but not for the co-injected or sham groups. Thus, quinolinic acid produced a significant deficit in performance of the radial maze task, and co-injection of kynurenic acid prevented this decline.

In the partially baited radial maze, two types of errors are possible within a session. Working memory errors occur when an arm of the baited set is re-entered after the food has been eaten, and reference memory errors occur when an arm of the never baited set is entered [21, 28]. The decrease in total correct responses from pre- to post-

surgery for the quinolinic acid injected group occurred as a result of increases in both mean ($\pm$S.E.M.) total reference memory errors (from 2.9 ± 0.6 to 4.9 ± 1.1) and working memory errors (from 0.1 ± 0.1 to 0.6 ± 0.3). However, with the small number of rats in this preliminary experiment, neither change achieved statistical significance.

The observation of a significant impairment of memory in the radial maze in rats with unilateral quinolinic acid lesions of the basal forebrain has not been reported previously, although unilateral injections of kainic acid have been found to produce this effect [5]. The present finding is in agreement with a previous study from this laboratory showing that quinolinic acid lesions impair memory in a T-maze alternation task [6] and with many previous studies showing that unilateral electrolytic [9, 23] or bilateral electrolytic, ibotenic or kainic acid lesions of the nbm [1, 4, 11, 12, 16, 19, 20, 22, 26, 27, 29] lead to memory deficits in a variety of tasks.

Results showed that co-injection of kynurenic acid with quinolinic acid prevented the neurotoxic effects of quinolinic acid on the cholinergic neurons of the basal forebrain. This was indicated by a failure to observe decreases in cortical ChAT activity, a finding in good agreement with previous reports [13, 14]. Moreover, kynurenic acid prevented memory deficits in the radial maze seen after quinolinic acid alone. These findings provide additional support for the hypotheses that cholinergic neurons of the nbm may be involved in the control of memory [3, 10]. Furthermore, they show that neuronal and memorial deficits produced by injection of the endogenous tryptophan metabolite, quinolinic acid, are prevented by co-injection of another endogenous tryptophan metabolite, kynurenic acid.

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