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Electrophysiological effects of exogenous and endogenous kynurenic acid in the rat brain: studies *in vivo* and *in vitro*

Review Article

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Summary. In this review, recent studies on the electrophysiological effects of de novo synthesized ("endogenous") kynurenic acid (KYNA) are discussed. Endogenous KYNA is normally formed as a byproduct of tryptophan metabolism. Evidence for a physiological role in neuronal excitability has not been strong, in part because brain levels are much lower than the K_D of KYNA at the glycine site of the NMDA receptor, where KYNA is thought to exert its most potent effect. The results suggest that, unexpectedly, even low concentrations of endogenous KYNA have physiological consequences. These levels of KYNA reduced the number of hippocampal slices with spontaneous epileptiform discharges after exposure to buffer lacking magnesium. However, effects on evoked responses to single afferent stimuli were not detected. Taken together, the data argue for a potentially important role of endogenous KYNA in suppression of seizure-like activity, and suggest a novel approach to anticonvulsant drug development that could have few side effects.

Keywords: Amino acids – Anticonvulsant – Entorhinal cortex – Glycine – Hippocampus – N-Methyl-D-aspartate – Tryptophan

Abbreviations: AMPA amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CNS central nervous system; EPSP excitatory postsynaptic potential; IPSP inhibitory postsynaptic potential; KYNA kynurenic acid; L-KYN L-kynurenine; NMDA N-methyl-D-aspartate

Introduction

KYNA is a staple of most neuroscience laboratories because of its well known antagonism of the NMDA and AMPA/kainate subtypes of glutamate

receptors. It is widely used to block fast glutamatergic transmission in order to examine phenomena mediated by other receptors (Stone and Burton, 1988; Herron and Malenka, 1994). At low concentrations, KYNA is a selective antagonist of the glycine co-agonist site of the NMDA receptor (K_D, approximately 8μM; Ganong and Cotman, 1986; Kessler et al., 1989; Parsons et al., 1997). Importantly, KYNA is also produced in the periphery and in the brain as a byproduct of the kynurenine pathway of tryptophan metabolism, albeit at relatively low concentrations (Stone, 1993; Brown, 1994). Although this pathway has been characterized in some detail, few studies have addressed the effects of endogenous KYNA on neurotransmission. This review summarizes experimental evidence that endogenous KYNA may indeed have physiological effects in the CNS.

Peripheral vs. central KYNA

One of the first questions that arose in assessing the potential role of endogenous KYNA in the CNS was whether KYNA could enter the brain from the circulation and have central effects. Peripheral KYNA is of particular importance because KYNA is produced most effectively in organs outside the brain (Stone and Connick, 1985; Brown, 1994). To address this question, KYNA was infused through a jugular catheter, and responses to afferent stimulation were recorded simultaneously in the hippocampus of urethane-anesthetized rats, using stereotaxic methods (Scharfman and Goodman, 1998). The hippocampus was chosen because its principal neurons receive well-characterized glutamatergic inputs (Storm-Mathisen and Ottersen, 1988) that can be activated relatively selectively, and because these neurons possess numerous NMDA receptors, with glycine sensitivity (Kleckner and Dingledine, 1989; Minota et al., 1989; McDonald et al., 1990).

Extracellular recordings were made either in the pyramidal cell layer of area CA1 in response to contralateral CA3 stimulation, or in the dentate gyrus granule cell layer in response to perforant path stimulation. In these experimental paradigms, a suprathreshold stimulus evokes action potentials in a number of neurons situated near the recording electrode, so that the recording electrode detects a negative voltage deflection (population spike; see arrow in Fig. 1) superimposed on a positive envelope (representing the sum of EPSPs and IPSPs in the same neurons; Lømo, 1971). Test responses were triggered intermittently (at 0.03 Hz) before drug infusion, and periodically thereafter, to monitor changes in population spike amplitude (Scharfman and Goodman, 1998).

After ensuring stability of the response to a test stimulus, KYNA (300 mg/kg) was infused over 10 minutes. Controls received a similar volume of saline infused at the same rate and over the same period of time. While saline had little effect, infusion of KYNA rapidly inhibited population spike amplitude. The latency to onset of the effect was less than 2 minutes. This inhibitory effect appeared specific because there was no change in population spike

latency or the associated fiber volley. Specificity was also indicated by the lack of effect of KYNA on antidromic population spikes, which were tested by stimulating area CA3 and recording from the contralateral CA3 pyramidal cell layer (Scharfman and Goodman, 1998).

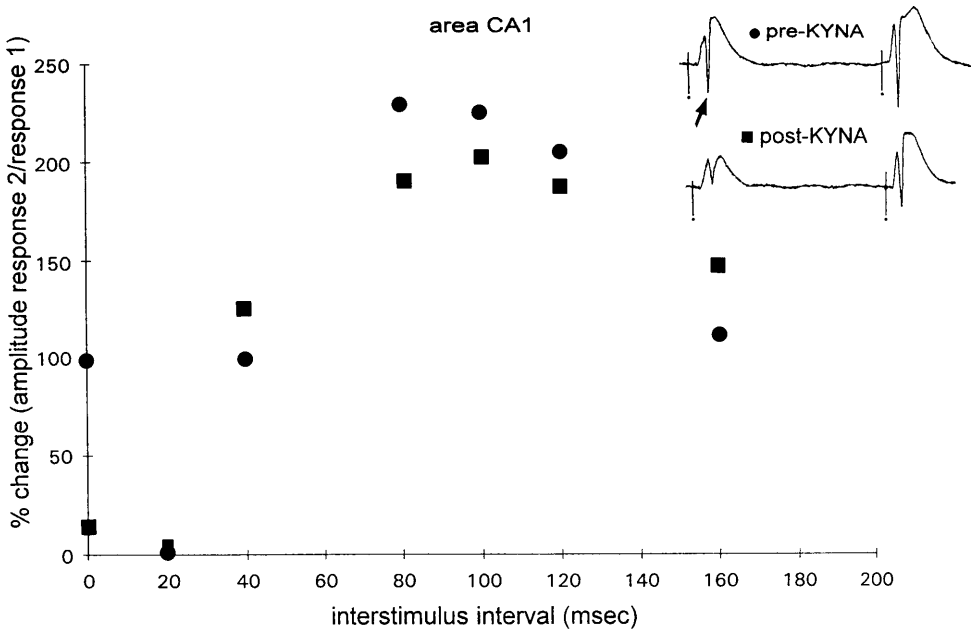
Interestingly, evoked responses recorded in the dentate gyrus (in response to its major glutamatergic afferent, the perforant path) were only weakly inhibited by the same dose of KYNA that strongly inhibited population spikes in area CA1 (Scharfman and Goodman, 1998; see also Fig. 1). Orthodromic responses recorded in area CA3 (in response to contralateral CA3 stimulation) were also relatively unaffected. The discrepancy between the effects in CA1 and the lack of effects in CA3 might be explained by the relative lack of NMDA binding in area CA3 (Monaghan and Cotman, 1985). NMDA receptors with different glycine sensitivity have been demonstrated in different neurons within rat parietal cortex (Kew et al., 1998), and this may also occur in hippocampus. Indeed, there are differences in the isoforms of the NMDA receptor subunit NR1 within the hippocampus (Zukin and Bennett, 1995), as well as differences in NR2 subunit mRNA and protein expression (Monyer et al., 1992, 1994; Watanabe et al., 1993; Jacobson and Cottrell, 1993; Sakurai et al., 1993; Petralia et al., 1994). NR2 subunits are of particular interest because they confer varying degrees of sensitivity to glycine (Mishina et al., 1993; McBain and Mayer, 1994). However, strychnine-insensitive glycine binding is not significantly different in CA1, CA3 and the dentate gyrus (McDonald et al., 1990).

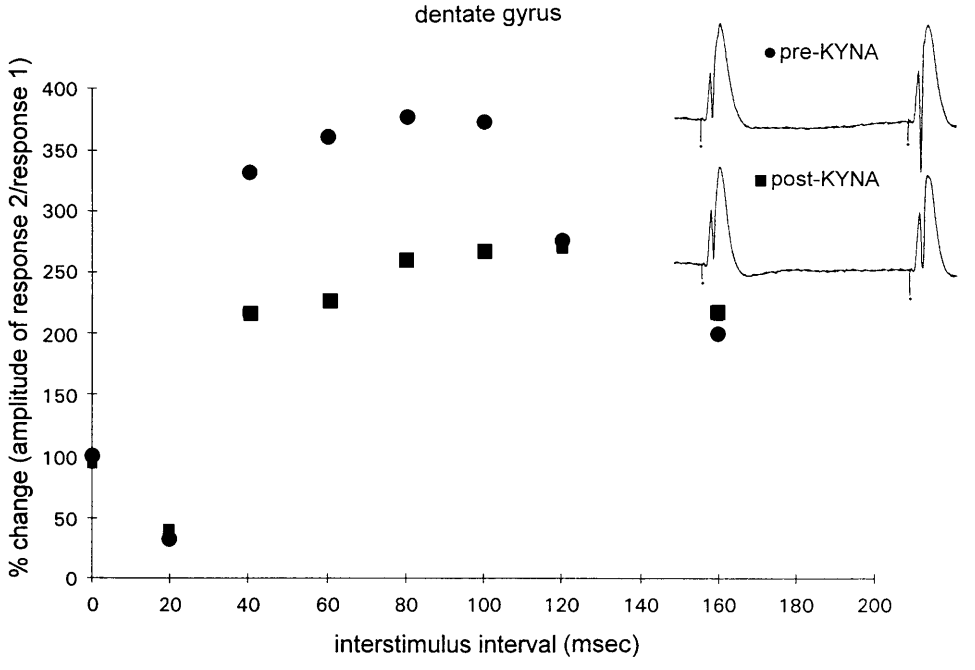
Another difference in the effects of KYNA between area CA1 and the dentate gyrus became apparent when pairs of stimuli were tested (Fig. 1). Intervals between pairs of stimuli were 20, 40, 70, 80, 100, 120, 160 and 200 msec. In the normal dentate gyrus, two identical stimuli delivered 40–100 msec apart produced paired pulse facilitation (i.e., the second stimulus produced a larger response than the first). Paired pulse facilitation is thought to be due to increased release of transmitter in response to the second stimulus because of residual calcium in the terminal following the first stimulus (Katz and Miledi, 1968), although other factors such as the waxing and waning of concurrent IPSPs may also play a role (Blundon et al., 1993; Metherate and Ashe, 1994). Consistent with the possible interference by concurrent inhibition in detecting NMDA receptor-associated effects, *in vivo* experiments with recording sites in posterior CA1, where facilitation was not evoked (presumably because of stronger inhibition in posterior hippocampus), showed no clear effects of KYNA. However, in all 4 experiments with relatively anterior recording sites in CA1, facilitation occurred, and subsequent administration of KYNA had a strong effect. As for dentate gyrus experiments, there was no effect of saline infusion on evoked responses in area CA1 (dentate, $n = 4$; CA1, $n = 3$).

In the dentate gyrus, KYNA had only weak effects on the response to the first stimulus (as described above), but substantially inhibited the response to the second stimulus (Fig. 1). The effect on the response to the second stimulus occurred when it was timed to fall within the window of paired pulse facilitation (40–100 msec interstimulus interval). Thus, KYNA decreased

paired pulse facilitation in the dentate gyrus preferential to an effect on the first evoked response. In contrast, KYNA strongly affected the response to both the first and second stimulus in area CA1 (Fig. 1).

One possible explanation for the differences between area CA1 and the dentate gyrus is that NMDA receptors are preferentially involved in





facilitation in the dentate gyrus relative to AMPA receptors (Joy and Albertson, 1993). In addition, facilitation might involve extrasynaptic NMDA receptors because the second stimulus of a pair would be likely to increase transmitter release and spillover outside the synaptic cleft (see Fig. 3). Conceivably, KYNA might have particularly strong effects on extrasynapic NMDA receptors.

It is notable that other studies of paired pulse responses in the dentate gyrus *in vitro* demonstrated equivalent effects on the first and second responses (Harris and Cotman, 1985). The difference between those results and ours could be due to the preferential stimulation of the medial perforant path fibers in the experiments of Harris and Cotman (1985). In our *in vitro* experiments, the lateral perforant pathway was activated preferentially. This could be important, because the degree of NMDA receptor activation may differ between the medial and lateral afferents to the dentate gyrus (Bramham and Sarvey, 1996).

Alternatively, differences in the effects of KYNA in area CA1 and the dentate gyrus could be explained by the different intrinsic characteristics of granule cells and CA1 pyramidal cells. Because granule cell resting membrane potentials are hyperpolarized relative to area CA1 pyramidal cells (Masukawa et al., 1982; Thompson et al., 1985; Scharfman, 1992; Staley et al., 1992, Williamson et al., 1993), granule cell responses to a single stimulus might normally have a very small NMDA receptor-mediated component. This follows from a greater voltage-dependent block by magnesium at more hyperpolarized potentials. NMDA receptors may become more important after a second stimulus, because in that case granule cell dendrites might be slightly depolarized (due to the residual EPSP after the first stimulus) or disinhibited (because of inhibition of GABA release following the activation of $GABA_B$ receptors).

Fig. 1. Effects of KYNA on responses to paired pulses with varying interstimulus intervals. *Top:* Responses of area CA1 pyramidal cells to contralateral CA3 stimulation *in vivo*. Responses of area CA1 pyramidal cells were examined before and after infusion of 300 mg/kg KYNA in a jugular catheter. The effects on the response to a single stimulus are shown at interstimulus interval "0". Note that KYNA greatly reduced the response to the first stimulus. The effects of KYNA on responses to the second stimulus of pairs are shown at the corresponding interstimulus interval. Thus, paired pulse inhibition was evident when the second stimulus was triggered 20 msec after the first. Paired pulse facilitation was maximal when intervals were 80–100 msec, and KYNA decreased paired pulse facilitation. All stimuli were the same intensity. Each symbol (circle, pre-KYNA; square, post-KYNA) represents the mean of 4 experiments. *Inset:* An example of evoked responses; the arrow points to the population spike. *Bottom:* Responses of dentate gyrus granules cells to angular bundle (perforant path) stimulation. Analogous to the area CA1 experiments, paired stimuli were tested in the dentate gyrus pre (circle) and post (square) infusion of KYNA. Note that there was little effect on the response to the first stimulus, unlike area CA1, but paired pulse facilitation was reduced. *Inset:* Exemplary dentate gyrus evoked responses, illustrating the relatively large effect of KYNA on the response to the second stimulus. Inset is modified from data shown in Scharfman and Goodman (1998)

Greater NMDA receptor activation would lead to a larger and longer lasting EPSP, and increased probability of reaching threshold. A larger response would also be expected upon slight depolarization because granule cell input resistance increases upon depolarization (Scharfman, 1994). In contrast, the normal resting potential of CA1 neurons is already depolarized.

In contrast to the effects of KYNA when the interstimulus interval was 40–100 msec, KYNA did not affect the second response when the interstimulus interval was brief (20 msec). Similar results were obtained in area CA1 and the dentate gyrus (Fig. 1). This could be due to the strong IPSPs that peak at 10–20 msec after a single stimulus, both in area CA1 and the dentate gyrus. The strong inhibition makes it difficult to detect an effect on a simultaneous NMDA receptor-mediated EPSP with extracellular recording. Indeed, under normal conditions, the second population spike is decreased, sometimes to 0, when the interstimulus interval is <30 msec ("paired pulse inhibition" or "paired pulse depression"). Paired pulse inhibition is thought to be a result of the actions of inhibitory neurons that are activated by the first stimulus and innervate neurons whose discharge comprises the population spike (Schwartzkroin and Mueller, 1987; Burdette and Gilbert, 1995a and b). It is not surprising that KYNA had little effect on paired pulse inhibition, because KYNA has no known actions at GABA receptors.

In vitro and *in vivo* approaches

The experiments described above were conducted in parallel *in vitro*, using the hippocampal slice preparation. This preparation provides the opportunity to test known concentrations of kynurenergic compounds directly, and to stimulate pathways similar to those that were activated *in vivo*.

Despite the difficulty in activating precisely the same pathways *in vivo* and *in vitro*, qualitatively similar potentials were evoked, with a similar time course and degree of paired pulse inhibition and facilitation. Notably, adding KYNA to the perfusing buffer ("bath-application") produced similar effects as those observed after KYNA infusion *in vivo*. Thus, 10 μM KYNA inhibited population spikes in area CA1 to approximately 81% of control, whereas in the dentate gyrus, 40 μM was required for this effect (Fig. 2). The relative effects of KYNA on paired pulse facilitation were also similar *in vivo* and *in vitro*, in that there was preferential inhibition of the second population spike in the dentate gyrus. Thus, 40 μM KYNA decreased the first population spike of a pair (interstimulus interval 70 msec) by approximately 84% and the second population spike by approximately 63% (Fig. 2).

Taken together, these data suggest that peripheral KYNA can enter the brain to some extent (cf. Fukui et al., 1991), and affect glutamatergic transmission to hippocampal neurons. Furthermore, 10–40 μM KYNA appear to be required to substantially decrease evoked responses. This much KYNA is unlikely to be supplied endogenously, since normal concentrations in rat are below 100 nM (Moroni et al., 1988). However, the KYNA concentration in the

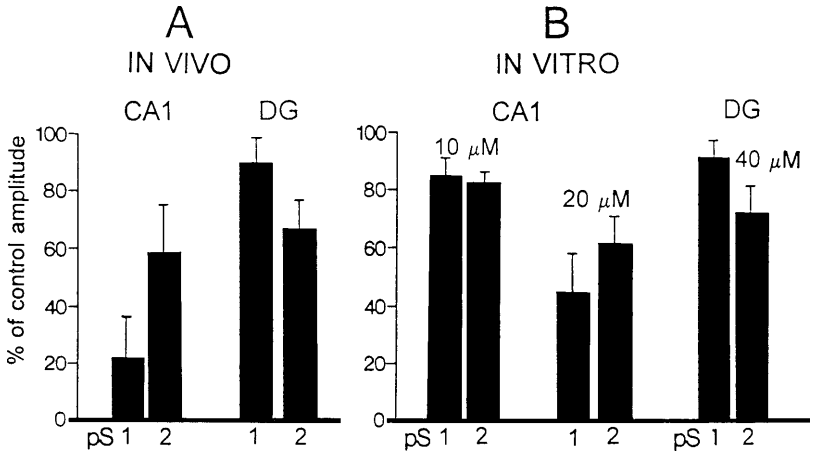


Fig. 2. Quantitation of KYNA effects in area CA1 and the dentate gyrus *in vitro* and *in vivo*. Means (± s.e.m.) are shown for data obtained *in vivo* (**A**) and *in vitro* (**B**). 300 mg/kg KYNA was infused over 10 min for *in vivo* experiments, and various concentrations (10–40 μM) were bath-applied *in vitro*. Measurements were made of the amplitude of the population spike evoked in response to the first stimulus (pS1) and second stimulus (pS2) using a 70–80 msec interstimulus interval. Data are from Scharfman and Goodman (1998)

human brain is approximately 1 μM (Turski et al., 1988). In circumstances that increase brain kynurenines, such as seizures (Baran et al., 1995; Wu et al., 1995; Löscher et al., 1996; Wu and Schwarcz, 1996), ischemia (Saito et al., 1993), infection (Heyes and Lackner, 1990; Moroni et al., 1991; Saito et al., 1992; Cheminal et al., 1996), or neurological disorders such as Down's syndrome (Baran et al., 1996) or Tourette's syndrome (Rickards et al., 1996), endogenous KYNA might in certain cases reach concentrations that are sufficient to modulate hippocampal synaptic transmission.

Effects of de novo-produced KYNA

To determine the possible physiological effects of de novo-produced KYNA, we applied its precursor, L-kynurenine (L-KYN), which is readily accumulated by astrocytes (Speciale and Schwarcz, 1990), and is then converted to KYNA (Turski et al., 1989; Swartz et al., 1990; Du et al., 1992; Guidetti et al., 1995; Fig. 3). Hippocampal slices were used to examine the effects of L-KYN treatment (for Methods see Scharfman and Ofer 1997). We predicted that L-KYN would be converted to KYNA and that newly formed KYNA would be released and then inhibit evoked responses in a similar manner as exogenously supplied KYNA. The amount of de novo-produced KYNA was determined fluorometrically (Swartz et al., 1990) in aliquots taken from the bath every 20 minutes.

Prior to L-KYN treatment, KYNA concentration was extremely low (Table 1), often below the level of detection. KYNA concentration slowly

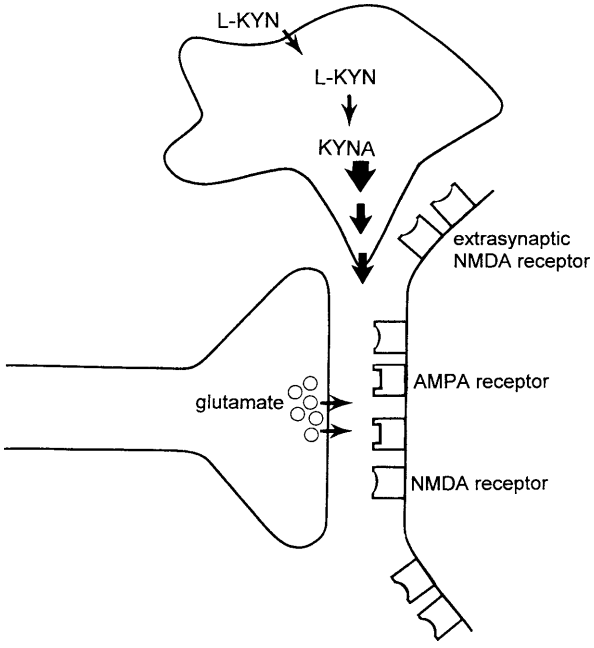


Fig. 3. Schematic diagram illustrating the focal release of *de novo*-produced KYNA from glia. Because L-KYN is converted to KYNA primarily in glia, KYNA may be released in a focal manner at synapses (large arrows), thus maximizing the KYNA concentration at NMDA receptors. If the same amount of KYNA were evenly distributed throughout the slice, a lesser concentration would be available at NMDA receptors. Extrasynaptic receptors could also be preferentially affected by KYNA because of their proximity to glia. However, the relative contribution of extrasynaptic receptors to synaptic transmission in the hippocampus and entorhinal cortex is presently unclear

rose during bath-application of L-KYN. In contrast, bath-application of KYNA itself produced a rapid rise in KYNA (Table 1).

Physiological recordings of evoked responses in area CA1, made between 0 and 30 minutes after the onset of L-KYN perfusion, indicated no effect of acute treatment on population spike amplitude and latency, even at the highest dose that was tested (200μM). The lack of effect prior to the production of detectable amounts of KYNA is consistent with previous studies which showed little effect of L-KYN itself on hippocampal neurons (Perkins and Stone, 1982). Moreover, responses examined 3 hours after onset of L-KYN infusion were still similar to controls, consistent with the requirement of 10μM KYNA or more to inhibit evoked responses in hippocampus (see above).

In order to evaluate the possible physiological effects of KYNA using another measure, the potential anticonvulsant action of KYNA was tested. We hypothesized that even if KYNA concentration was insufficient to inhibit evoked responses to single afferent stimuli, spontaneous epileptiform activity might nevertheless be inhibited.

Epileptiform activity was elicited by exposing slices to buffer lacking magnesium (Scharfman and Ofer, 1997). Seizure-like activity never occurs in slices perfused with regular buffer, but reliably follows 30–45 minutes perfusion with magnesium-free buffer. The epileptiform events are qualita-

Table 1. KYNA content in perfusate of slices exposed to L-KYN or KYNA

A. Biochemistry		
	KYNA (nM)	
Time (min)	200μM L-KYN (n = 5)	2μM KYNA (n = 4)
−20	9 ± 6	3 ± 1
0	13 ± 5	10 ± 3
20	270 ± 75	1,305 ± 412
40	388 ± 118	1,447 ± 196
60	484 ± 153	1,566 ± 220
80	552 ± 174	1,558 ± 216
100	649 ± 197	1,577 ± 218
120	608 ± 202	1,567 ± 229
140	638 ± 196	1,718 ± 193
160	754 ± 244	1,356 ± 221
180	821 ± 261	1,256 ± 104
B. Physiology		
CA3*	45.1% (n = 23/51)	67.2% (n = 29/43)
EC*	37.2% (n = 18/51)	78.3% (n = 36/46)

A. Biochemistry: Mean KYNA concentration (± s.e.m.), determined from samples of the buffer solution surrounding slices, is listed as a function of time. Compounds were added at time 0. At 40 min, buffer was switched to one that lacked magnesium. The same concentrations of L-KYN or KYNA were continued until 180 min, when slices were examined for spontaneous activity in the CA3 pyramidal cell layer and layer V of the entorhinal cortex.
B. Physiology: The mean percent of slices exhibiting spontaneous activity at 180 min is listed for area CA3 and the entorhinal cortex (EC). * = differences between L-KYN and KYNA were statistically significant ($p < 0.05$, t-test). In each experiment, 12–14 slices from one animal were placed in the recording chamber. Sample size (n) refers to the total number of slices exhibiting spontaneous epileptiform activity at 180 min out of the total number of slices exhibiting robust responses to single afferent stimuli (hilar stimulation, CA3; white matter stimulation, EC).

tively similar from slice to slice, and consist of spontaneous, repetitive field potentials that can be recorded extracellularly (Anderson et al., 1986; Walther et al., 1986; Jones and Heinemann, 1990; Jones and Lambert, 1990). In area CA3, brief (50–250 msec) bursts of population spikes occur at a frequency of 0.1–3 Hz on a large (5–10 mV) positive envelope, sometimes accompanied by 1–2 afterdischarges. In the entorhinal cortex, 200–500 msec periods of repetitive negative potentials occur after perfusion with buffer lacking magnesium. They can be recorded as negative potentials in layers II-IV, whereas population spikes and mixed positive-negative field potentials are recorded in deeper layers. These events are less regular than those in area CA3, occurring at 0.05–0.1 Hz.

To test the effects of KYNA, slices were pretreated with 200μM L-KYN for 40 minutes, a time sufficient for KYNA formation (Table 1). Then a buffer was perfused that included L-KYN, but contained no magnesium. L-KYN

treatment had a distinct anticonvulsant effect, yielding fewer slices with spontaneous activity in both area CA3 and the entorhinal cortex (Table 1; Scharfman and Ofer, 1997). Surprisingly, recent experiments have shown that even low concentrations of KYNA ($<10\mu$M) have anticonvulsant effects (Scharfman et al., 1999).

Further comparison between L-KYN and KYNA indicated that de novo-produced ("endogenous") KYNA was in fact more effective than exogenously-supplied KYNA. 200μM L-KYN and 2μM KYNA were compared because similar KYNA concentrations were measured after these two treatments (Table 1). Slices were first pretreated with L-KYN or KYNA for 40 minutes and then exposed to buffer lacking magnesium. Slices exposed to 200μM L-KYN had less spontaneous epileptiform activity than slices treated with 2μM KYNA, even though the KYNA concentration was somewhat lower in the L-KYN group than the KYNA group (Table 1). This difference could be due to the fact that de novo-produced KYNA is formed preferentially in glia (Du et al., 1992; Guidetti et al., 1995), which could release the enzymatic product in a focal, concentrated manner at NMDA receptors (Fig. 3).

The experiments also revealed a different effect of KYNA in area CA3 and the entorhinal cortex, the latter being more sensitive to low levels of KYNA. This is not surprising given that the epileptiform activity in the entorhinal cortex induced by perfusion with low magnesium buffer is blocked completely by NMDA antagonists (Jones and Heinemann, 1990; Jones and Lambert, 1990), but NMDA antagonists are only partially effective in area CA3 (Brady and Swann, 1988; Stone, 1988; Lee and Hablitz, 1990). One can speculate that these regional differences might be due to a greater density of NMDA receptors, or different subunit composition of receptors, in the entorhinal cortex.

Conclusions

The *in vivo* and *in vitro* experiments reviewed and illustrated here lead to the following conclusions and predictions: first, peripheral KYNA can enter the brain to a certain extent and subsequently produce central effects. This ability to penetrate into the CNS might be enhanced when the blood-brain barrier is compromised (e.g. during seizures), allowing blood-derived KYNA to influence neuronal excitability.

Second, neurons in different brain regions (for example the hippocampus and the entorhinal cortex) or within a brain area (i.e., CA1 vs. the dentate gyrus) differ in their sensitivity to KYNA. Thus, entorhinal cortical neurons are highly sensitive to KYNA. Area CA1 pyramidal neurons appear to be considerably more sensitive than dentate granule cells, at least for the pathways that were examined here. Interestingly, the sensitivity of CA1 neurons is greater than noted in previous studies, perhaps because we considered KYNA concentrations that reduced but did not totally block evoked responses. Conceivably, these differences, which might be caused by

variations in the composition and distribution of NMDA receptors, could be exploited for targeting certain neuronal populations by drugs that raise brain KYNA levels.

Third, manipulation of L-KYN, the precursor of KYNA, can be used to elicit effects which appear to be identical to those of KYNA. This can be achieved either by administration of L-KYN itself, or by pharmacological interference with kynurenine pathway metabolism (Moroni et al., 1991; Speciale et al., 1997).

Fourth, de novo formation of KYNA is an efficient means to prevent epileptogenesis, dampening spontaneous discharge with little effect on normal synaptic transmission. This may provide an attractive, novel approach for the development of anticonvulsant agents.

Finally, comparison of the physiological effects of exogenous and newly formed KYNA demonstrates that past measurements of tissue or extracellular levels of KYNA in the brain may have substantially underestimated KYNA concentrations. Thus, the submicromolar levels of brain KYNA reported by us and others (Moroni et al., 1988; Turski et al., 1988; Swartz et al., 1990) did not take into account that dilution occurs due to either tissue homogenization or sampling of the extracellular compartment distant from the synapse.

Taken together, these findings and considerations justify further efforts to explore the potential role of endogenous KYNA as a modulator of normal and abnormal synaptic transmission.

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