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Glutamate and Kynurenate in the Rat Central Nervous System Following Treatments with Tail Ischaemia or Diclofenac

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Abstract

Kynurenate is an endogenous antagonist at the allosteric glycine site on the *N*-methyl-D-aspartate (NMDA) receptor, and may have a role in ameliorating nociceptive processes through modulation of NMDA receptor function. While antinociceptive effects of non-steroidal anti-inflammatory drugs (NSAIDs) are mediated peripherally and possibly centrally through inhibition of prostaglandin synthesis, there is also evidence for centrally mediated prostaglandin-independent antinociceptive effects that may result from increased central nervous system (CNS) concentrations of kynurenate. We have investigated the effects of the NSAID diclofenac, (40 mg kg^{-1}, s.c.; administered to rats 1 h before killing) or the exposure of rats to noxious stimulation (tail ischaemia for 20 min before killing), on the concentrations of glutamate and kynurenate in discrete CNS regions.

Regional CNS tissue concentrations of diclofenac were between 3·0–3·8 nmol g^{-1}. The corresponding regional glutamate concentrations ranged between 4·8–10·6 μmol g^{-1}, and were significantly lower in the ischaemia group when compared with both control (15%, $P < 0{\cdot}05$) and diclofenac-treated (19%, $P < 0{\cdot}002$) groups. Kynurenate concentrations in these CNS regions ranged between 3·3–45·8 pmol g^{-1}. Pairwise comparisons between the control and diclofenac-treated groups found significant increases in kynurenate concentrations in the diencephalon and lumbo-sacral regions of the CNS ($P = 0{\cdot}05$).

Noxious stimulation from tail ischaemia appeared to be associated with increased release of glutamate. Additionally, NSAIDs appeared to increase kynurenate concentrations in the spinal cord and diencephalon. Antagonism by kynurenate of glutamate effects at NMDA receptors may contribute to the antinociceptive effects of NSAIDs.

Non-steroidal anti-inflammatory drugs (NSAIDs) are believed to exert their antinociceptive and anti-inflammatory effects by inhibition of prostaglandin synthesis in peripheral injured tissues (Vane 1971; Ferreira et al 1972, 1973). Subsequent work has found antinociceptive effects of NSAIDs after both central and peripheral administration (Ferreira et al 1978). More recent analyses have found evidence for a dissociation between their anti-inflammatory and antinociceptive effects (McCormack & Brune 1991). There is also evidence supporting a prostaglandin-independent central mechanism of action for NSAIDs from studies of the differential effects of individual flurbiprofen enantiomers on prostaglandin synthesis and nociception (Brune et al 1991; Geisslinger et al 1994).

N-Methyl-D-aspartate (NMDA) receptors are an important site of action for glutamate (Monaghan et al 1989) and appear to play an important role in the central sensitization that follows intense nociceptor stimulation (Davies & Lodge 1987; Dickenson & Sullivan 1987). The tryptophan metabolite, kynurenate, acts as a broad-spectrum excitatory amino acid antagonist (Perkins & Stone 1982; Ganong et al 1983), but demonstrates a selective affinity for the NMDA receptor (Ganong & Cotman 1986). Antagonism of the NMDA receptor by kynurenate is mediated by its binding to the glycine site (Birch

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et al 1988; Danysz et al 1989). The activation of the glycine site on the NMDA receptor has been shown to be a requirement for the development of central sensitization in the spinal cord (Dickenson & Aydar 1991).

Antinociceptive effects of kynurenate have been demonstrated in-vivo (Yaksh 1989; Hajos & Engberg 1990; Nasstrom et al 1992). In other work, pharmacologically induced increases in kynurenate concentrations in the rat brain have been associated with modulation of NMDA receptor function (Russi et al 1992). Following peripheral administration of the biosynthetic precursor of kynurenate, L-kynurenine, to rats, increased brain concentrations of kynurenate were found that appeared to result from the metabolism of kynurenine to kynurenate within the brain (Swartz et al 1990a). It has been postulated that NSAIDs may indirectly modulate NMDA receptor function in the CNS by altering the activity of hepatic tryptophan-2,3-dioxygenase and increasing the biosynthesis and hepatic output of kynurenine. CNS uptake of kynurenine and metabolism to kynurenate may act to reduce NMDA receptor mediated central sensitization (McCormack 1994).

In this study, the effects of two treatments on regional CNS tissue levels of glutamate and kynurenate have been investigated in the rat. The first treatment, tail ischaemia (shown to induce hyperalgesia that is antagonized by NSAIDs (Gelgor et al 1986)) was induced for a 20-min period before the rats were killed. The second treatment was a dose of the NSAID diclofenac (40 mg kg^{-1}, s.c.) administered 60 min before the rats were killed.

Materials and Methods

Compounds

α-Aminoadipic acid, L-glutamic acid, ketoprofen, kynurenic acid, sodium diclofenac, tetrabutylammonium hydrogen sulphate (TBAHS), *N*, *N*-diisopropylethylamine (N,N-DIPEA), and pentafluorobenzyl bromide were purchased from Sigma (St Louis, MO).

Experimental

The protocol was approved by the local Animal Care and Ethics Committee. Male Sprague–Dawley rats (210–280 g) were housed at 20°C on a 12-h light–dark cycle with free access to food and water until the day of the study. They were divided into three groups (n = 6). The first group acted as the control and was killed without receiving any treatment. The second group was subjected to the noxious stimulus of tail ischaemia as previously described by Gelgor et al (1986). A cuff was applied to the base of the rat tail, inflated to 240 mmHg for 20 min, then deflated; the rats were immediately killed. The third group was administered diclofenac (sodium salt, 1% w/v, 40 mg kg^{-1}, s.c.), 1 h before being killed.

The animals were killed by asphyxiation with CO_2, then immediately decapitated. The brain and spinal cord were rapidly removed. Blood was washed from the brain surface and the tissue rapidly chilled by immersion in ice-cold saline. The dura and associated blood vessels were removed before regional dissection on ice. First, the pons–medulla and cerebellum were separated from the midbrain by a coronal incision at the base of the cerebral peduncles. A second incision was made in the coronal plane at the level of the optic chiasm to separate the midbrain from the hypothalamus. Finally, the temporal lobes and amygdala were separated from the ventral surface of the hypothalamus, and the diencephalon extirpated from the cortical shell to the level of the corpus callosum. The cortical shell was subsequently referred to as cortex. The spinal cord was rinsed with ice-cold saline, and the dura was removed before division into brachio–thoracic and lumbo–sacral segments. Tissue samples were immediately frozen by immersion in a slurry of dry ice and ethanol, and stored for not longer than three months at −80°C until the time of analysis.

Tissue was prepared for analysis by homogenization into 5–10 vol per tissue weight of aqueous formic acid (5 M) in methanol (20% v/v). Larger volumes of cortical homogenate were placed in 10-mL polypropylene centrifuge tubes and centrifuged (3000 rev min^{-1}, 20 min), while smaller volumes of tissue homogenates from the other CNS regions were placed in 2-mL polypropylene microfuge tubes and centrifuged (11 000 rev min^{-1}, 20 min). The resulting supernatants were decanted with a Pasteur pipette. A sample of supernatant (100 μL) was taken for analysis of glutamate and diclofenac by GC–MS; the remainder (0·3–1·0 mL) was transferred into a glass vial (2 mL), dried under N_2, and reconstituted in $HClO_4$ (0·1 M, 200 μL) before storage at −80°C until analysis of kynurenate by HPLC.

Determination of glutamate and diclofenac by GC–MS

Tissue concentrations of glutamate and diclofenac were determined by GC–MS using pentafluoro-

benzylation in the presence of TBAHS and the base catalyst N,N-DIPEA. The method was developed from a previously described technique for the quantification of tryptophan metabolites (Naritsin et al 1995).

The internal standard solution contained α-aminoadipate (1 mM) and ketoprofen (10 mM) in aqueous formic acid (5 M) in methanol (20% v/v). In the final method, internal standard solution (100 μL) and TBAHS (40 μL, 100 μM), also dissolved in aqueous formic acid (5 M) in methanol (20% v/v), were added to CNS tissue supernatant (100 μL) in glass vials (2 mL). Vials were briefly vortex-mixed before being dried under N_2, and preheated without caps (65°C, 10–15 min) before addition of the derivatizing reagent. To optimize the formation of derivatives with pentafluorobenzyl bromide the procedure was performed with increasing quantities of added TBAHS and different times were allowed for derivatization. This was done using a standard solution in aqueous formic acid (5 M) in methanol (20% v/v) and a supernatant derived from whole brain homogenized into 5 vol per tissue weight of aqueous formic acid (5 M) in methanol (20% v/v). The standard solution contained 100 μM concentrations of test compounds and equivalent concentrations were added to whole brain homogenate.

The derivatizing reagent, prepared immediately before use, contained pentafluorobenzyl bromide (2% v/v) and N,N-DIPEA (5% v/v) in acetonitrile pre-dried by the addition of anhydrous K_2CO_3. Derivatizing reagent (200 μL) was added to each vial, and the vials capped before being briefly vortex-mixed and heated (65°C, 1 h). After cooling, H_2SO_4 (300 μL, 1 M) and toluene (500 μL) were added, and the vials were vortex-mixed (15 s). The organic and aqueous phases were separated by centrifugation (1500 rev min^{-1}, 5 min), and samples (200 μL) of the toluene extract were placed into polypropylene inserts (250 μL) for analysis by GLC–MS.

Toluene extracts (1 μL) were analysed on a Hewlett Packard 5972 MS/5890 series II GLC–MS system after injection onto an HP-5MS capillary column (30 m × 0·25 mm i.d., film thickness 0·25 μm), using a temperature program (150°C for 2 min, followed by 50°C min^{-1} to 270°C). The electron energy was 70 eV. Helium was used as the carrier gas (flow rate 1 L min^{-1}). The following ions (and their retention times) were monitored with a dwell time of 100 ms: glutamate m/z 264 (5·8 min), ketoprofen m/z 209 (7·4 min), diclofenac m/z 214 (8·2 min), and α-aminoadipate m/z 476 (9·0 min).

Determination of kynurenate by HPLC

Tissue concentrations of kynurenate were determined by a modified HPLC procedure reported by Swartz et al (1990b). Separation was performed on a C18 column (3·9 × 150 mm, 4 μm, Nova-Pak). The mobile phase was delivered by a Shimadzu LC-10AD pump (flow rate of 1 mL min^{-1}) and contained sodium acetate (50 mM, pH 6·3), TBAHS (1 mM), and acetonitrile (7·5% v/v). Zinc acetate (0·5 M) was delivered as a post-column reagent (flow rate 0·5 mL min^{-1}) by a Waters 510 HPLC pump. Column eluate fluorescence was measured, with a Shimadzu RF-10A detector, using excitation and emission wavelengths of 246 and 395 nm, respectively.

Frozen samples of $HClO_4$-treated CNS tissue residue were allowed to thaw (30 min) before analysis, vortex-mixed briefly, transferred to polypropylene tubes (1·5 mL), and centrifuged (7000 rev min^{-1}, 5 min). Supernatants were transferred to polypropylene inserts (250 μL) for analysis; 50-μL samples were injected onto the column.

Data analysis

Statistical analyses were performed using Statistix (version 4, Analytical Software, Tallahassee, FL). Normality of the data was tested using the Wilk–Shapiro statistic. Tissue concentrations of glutamate were analysed by two-factor analysis of variance with post-hoc pairwise comparisons performed by the method of least significant differences (LSD). Diclofenac concentrations were analysed by single-factor analysis of variance. Tissue concentrations of kynurenate were analysed by Kruskal–Wallis analysis of variance, and individual comparisons performed using the Mann–Whitney U test.

Results

Derivatization conditions

The effect of increasing quantities of TBAHS on the formation of derivatives with pentafluorobenzyl bromide for a standard solution and whole brain homogenate is shown in Figure 1. The optimal amount of TBAHS for derivatization of all compounds appeared to be 4 μmol/100 μL solution, and optimal heating conditions for derivatization were found to be 65°C for 1 h. These were used in the final method.

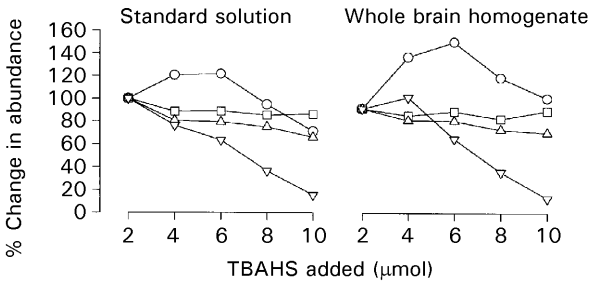

Figure 1. The effect of increasing amounts of tetrabutylammonium hydrogen sulphate (TBAHS) on the formation of pentafluorobenzyl derivatives for a 100 μM standard solution of aqueous formic acid (5 M) in methanol (20 % v/v), containing glutamate (○), ketoprofen (□), diclofenac (△), and α-aminoadipate (▽) or whole brain homogenate to which has been added the same concentration with each compound.

GLC–MS glutamate/diclofenac assay characteristics

Standards containing increasing concentrations of glutamate and diclofenac were prepared in pooled supernatant derived from homogenization of whole rat brains into 5 vol per tissue weight of aqueous formic acid (5 M) in methanol (20% v/v). The slope and r^2 values of the associated standard curves ($n = 9$) were $2{\cdot}82 \pm 0{\cdot}19$ and $0{\cdot}986 \pm 0{\cdot}005$ ($P < 0{\cdot}001$), respectively, for glutamate (0·5–5 mM) while, for diclofenac (0·2–5 μM), the values were $0{\cdot}105 \pm 0{\cdot}002$ and $0{\cdot}997 \pm 0{\cdot}002$ ($P < 0{\cdot}001$), respectively. The interassay coefficient of variation was 12% for glutamate and 6% for diclofenac. A signal-to-noise ratio of 20 : 1 was found for diclofenac following injection of 0·02 pmol onto the column. However, the signal for glutamate was no longer linear below amounts of 0·5 pmol injected onto the column, although the signal-to-noise ratio for 0·5 pmol was 15 : 1. Standard solutions of kynurenate were linear for an m/z of 325 between 0·05–5 pmol ($r^2 = 0{\cdot}997$, $P < 0{\cdot}001$), and the signal-to-noise ratio for 0·05 pmol injected onto the column was 5 : 1. It was not possible to load sufficient material onto the column to detect a signal for kynurenate in toluene extracts of derivatized whole brain supernatant, hence the separate HPLC assay needed to be developed.

HPLC kynurenate assay characteristics

The minimum level of sensitivity for kynurenate, at a signal-to-noise ratio of 2 : 1, was for 50 fmol injected onto the column, and the signal was linear ($r^2 = 0{\cdot}997$, $P < 0{\cdot}001$) over a concentration range of 1–200 nM at an injection volume of 50 μL. Standard curves ($n = 6$), prepared by adding increasing concentrations of kynurenate (10–100 nM) to pooled whole brain supernatant, prepared as above, gave respective slopes and r^2 values of $2{\cdot}89 \pm 0{\cdot}13$ and $0{\cdot}9989 \pm 0{\cdot}0004$ ($P < 0{\cdot}001$), respectively. The interassay coefficient of variation was 15%.

Recovery studies

Recovery was studied by homogenizing whole rat brain into 5 vol per tissue weight of aqueous formic acid (5 M) in methanol (20% v/v). Known amounts of each compound were added, and the amount of each compound in the supernatant after centrifugation was subsequently determined and compared with the amount originally added (Table 1).

Effects of ischaemia or diclofenac on CNS tissue glutamate concentrations

Two-factor analysis of variance found a significant effect of both treatment ($P < 0{\cdot}01$) and region ($P < 0{\cdot}0001$) on CNS tissue glutamate concentrations (Table 2). Although no significant differences were apparent from pairwise comparisons of treatments, overall comparison of regions between treatment groups found that tissue concentrations were significantly lower in the ischaemia group than in the control (15%, $P < 0{\cdot}05$) and diclofenac-treated (19%, $P < 0{\cdot}002$) groups. There were significant regional differences ($P < 0{\cdot}001$) in tissue concentrations within treatments when cortex, diencephalon, and cerebellum were compared with brachio–thoracic and lumbo–sacral regions. Pairwise comparisons of regional glutamate concentrations within each treatment found that the regional differences were larger in the control

Table 1. Percentage recovery values for glutamate, α-aminoadipate, diclofenac, kynurenate, and ketoprofen from whole brain homogenized, at 5 vol per tissue weight, into 5 M aqueous formic acid in methanol (20% v/v), after the addition of known concentrations of each compound.

Compound	Concentration	Recovery
Glutamate	1·0 mM	$45{\cdot}6 \pm 0{\cdot}8$
	2·5 mM	$60{\cdot}9 \pm 2{\cdot}4$
	5·0 mM	$73{\cdot}6 \pm 1{\cdot}9$
α-Aminoadipate	0·1 mM	$89{\cdot}7 \pm 0{\cdot}8$
	0·5 mM	$71{\cdot}7 \pm 1{\cdot}0$
	1·0 mM	$66{\cdot}2 \pm 0{\cdot}9$
	2·0 mM	$59{\cdot}2 \pm 2{\cdot}2$
Diclofenac	0·1 μM	$95{\cdot}7 \pm 3{\cdot}0$
	1·0 μM	$91{\cdot}8 \pm 2{\cdot}5$
	10·0 μM	$87{\cdot}5 \pm 1{\cdot}2$
Ketoprofen	1·0 μM	$91{\cdot}8 \pm 2{\cdot}5$
	10·0 μM	$87{\cdot}5 \pm 1{\cdot}2$
Kynurenate	10·0 nM	$70{\cdot}0 \pm 6{\cdot}9$
	20·0 nM	$53{\cdot}7 \pm 4{\cdot}2$
	100·0 nM	$52{\cdot}7 \pm 2{\cdot}8$

Values are shown as the mean ± s.e. ($n = 4$).

Table 2. Concentrations of glutamate and kynurenate in CNS tissue from groups (n = 6) of control, diclofenac-treated, and tail ischaemia-treated rats.

CNS tissue	Glutamate: mean (and standard deviation) (μmol (g tissue)$^{-1}$)		
	Control	Diclofenac	Ischaemia
Cortex	9·5 (1·7)	9·2 (2·2)	8·1 (1·4)
Diencephalon	10·1 (2·7)	10·6 (3·7)	8·2 (1·8)
Midbrain	8·2 (2·2)	7·9 (3·2)	6·7 (1·2)
Cerebellum	8·4 (3·1)*	8·6 (3·1)	7·0 (1·2)
Pons–medulla	5·9 (1·2)[1]	6·5 (2·3)[2]	5·0 (0·4)[3]
Brachio–thoracic cord	5·1 (1·6)*[1]	6·4 (2·6)[2]	4·8 (1·0)[3]
Lumbo–sacral cord	5·5 (1·6)[1]	6·5 (2·8)[2]	5·3 (1·5)
	Kynurenate: median (and quartile) (pmol (g tissue)$^{-1}$)		
CNS tissue	Control	Diclofenac	Ischaemia
Cortex	3·8 (1·5)*	4·6 (3·3)	3·3 (2·1)
Diencephalon	4·2 (0·3)*	8·2 (60·0)[4]	3·8 (2·1)
Midbrain	13·6 (6·2)	14·9 (6·2)	19·1 (20·4)
Cerebellum	7·7 (3·4)*	9·3 (4·9)	7·6 (5·8)
Pons–medulla	27·5 (21·7)	22·3 (15·7)	13·9 (27·2)
Brachio–thoracic cord	14·0 (11·4)*	28·0 (12·5)[5]	12·6 (17·5)
Lumbo–sacral cord	18·2 (10·8)	45·8 (21·9)[4,5]	12·8 (8·8)[6]

*n = 5; [1]$P < 0{\cdot}005$ vs cortex and diencephalon; [2]$P < 0{\cdot}005$ vs diencephalon; [3]$P < 0{\cdot}05$ vs cortex and diencephalon; [4]$P < 0{\cdot}05$ diclofenac vs control; [5]$P < 0{\cdot}025$ diclofenac cortex vs diclofenac brachio–thoracic and lumbo–sacral; [6]$P < 0{\cdot}05$ ischaemia cortex vs ischaemia lumbo–sacral.

($P < 0{\cdot}005$) and diclofenac ($P < 0{\cdot}005$) group than in the ischaemia injury group ($P < 0{\cdot}05$).

Diclofenac concentrations in CNS tissue

There were no significant differences in the concentrations of diclofenac between the various CNS regions 1 h after the administration of diclofenac (Table 3).

Effects of ischaemia or diclofenac on CNS tissue kynurenate concentrations

While the data for CNS tissue concentrations of glutamate exhibited normality (Wilk–Shapiro statistic = 0·966), those for kynurenate were not normally distributed (Wilk–Shapiro statistic = 0·566). Consequently the kynurenate tissue concentration data were analysed by non-parametric measures (Table 2). Significant effects were found for treatment ($P < 0{\cdot}05$) and region ($P < 0{\cdot}01$) (Kruskal–Wallis analysis of variance). Subsequent pairwise comparisons of the mean ranks for treatment and region found a significant difference between diclofenac and ischaemia treatments ($P < 0{\cdot}05$), and between cortex and brachio–thoracic regions ($P < 0{\cdot}05$). Pairwise Mann–Whitney U comparisons found significant differences ($P < 0{\cdot}05$) between control and diclofenac groups in the diencephalon and lumbo–sacral regions. Significant regional differences were also found in the diclofenac group ($P < 0{\cdot}025$) when cortex was compared with brachio–thoracic and lumbo-sacral regions, and in the ischaemia group ($P < 0{\cdot}05$) between cortex and lumbo–sacral regions, but no significant regional differences were found in the control group.

Table 3. Mean (and standard deviation) concentrations of diclofenac in CNS tissue of rats (n = 6) 60 min after the subcutaneous administration of 40 mg kg^{-1} diclofenac.

Region	Diclofenac (nmol (g tissue)$^{-1}$)
Cortex	3·0 (0·9)
Diencephalon	3·2 (0·8)
Midbrain	3·0 (1·0)
Cerebellum	2·6 (0·8)
Pons–medulla	3·1 (1·1)
Brachio–thoracic	3·7 (1·2)
Lumbo–sacral	3·8 (1·0)

Discussion

The results support a possible involvement of the NSAID diclofenac in prostaglandin independent CNS mechanisms of antinociception by increasing regional CNS concentrations of kynurenate. It is noted that a wide range of values for kynurenate was found here and this was also found in the work of Swartz et al (1990a). As this variability is much greater than that for either glutamate or diclofenac,

it could be related to variability in the kinetics of the precursor substance kynurenine.

A low recovery for glutamate was found at a 1·0 mM concentration added to whole brain homogenate, but recovery increased at higher concentrations. The endogenous concentration of glutamate in whole brain supernatant was $1{\cdot}34 \pm 0{\cdot}03$ mM ($n = 7$), and this high endogenous concentration was probably the cause of the lower recovery for 1·0 mM added glutamate. For the remaining compounds, the efficiency of extraction generally increased as the concentration added to whole brain homogenate decreased.

It is generally believed that the distribution of NSAIDs into CNS tissue is low due, principally, to their avid binding to soluble plasma proteins (Dehouck et al 1992; Davies & Anderson 1997). Diclofenac is extensively protein bound in plasma ($> 99{\cdot}7\%$) and its concentrations in cerebrospinal fluid were found to be only 8·2% of those in plasma suggesting poor distribution into the CNS (Davies & Anderson 1997). In this study, CNS tissue concentrations of diclofenac 60 min after a subcutaneous 40 mg kg^{-1} dose were between 3·0–3·8 nmol g^{-1}. We have found that this dose was necessary to suppress the behavioural response to the ischaemic injury and that plasma concentrations sampled at the same time following this dose of diclofenac were 81 ± 8 nmol mL^{-1} ($n = 6$) (unpublished observations). The brain diclofenac concentrations at 60 min were therefore $\sim 4\%$ of those present in plasma, confirming the general belief that there is poor distribution of diclofenac across the blood–brain barrier.

This investigation found a general reduction in regional CNS tissue concentrations of glutamate immediately following tail ischaemia, with concentrations comparable with those found in other work where subchronic administration of neuroleptics altered CNS tissue concentrations of glutamate (Lindfors et al 1987). Tail ischaemia generates a noxious stimulus that induces a period of hyperalgesia (Gelgor et al 1986). Increased extracellular concentrations of glutamate, consistent with an increase in release, have been found following acute nociceptive stimulation of the rat dorsal spinal cord (Skilling et al 1988). The decreases in regional CNS tissue concentrations of glutamate immediately following tail ischaemia are consistent with increased release and metabolism of glutamate following re-uptake into nerve terminals and glial cells.

Regional CNS tissue kynurenate concentrations found in this study were also comparable with those reported by Moroni et al (1988). Kynurenate concentrations were greater in the diencephalon and lumbo–sacral regions of the diclofenac-treated than of the control group. Spinal kynurenate concentrations in the diclofenac-treated group were significantly higher than the concentrations in the cortex, but there was no significant difference between these regions in the control group. This suggests diclofenac may have increased the synthesis of kynurenate in these regions. Similarly, the difference in kynurenate concentrations, found between cortex and lumbo–sacral regions in the ischaemia group, suggests this noxious stimulus may have increased the synthesis of kynurenate in spinal tissue. However, from this preliminary study with one dose and one sampled time point, it is not possible to determine whether diclofenac acted directly on CNS tissue to increase kynurenate synthesis or altered the uptake of its precursor, kynurenine, secondary to an increase in hepatic output of kynurenine.

Tryptophan is converted to the biosynthetic precursor of kynurenate, kynurenine, by the action of the enzyme tryptophan 2,3-dioxygenase in the liver and indolamine 2,3-dioxygenase in the brain (Stone 1993). In contrast to kynurenine, which appears to readily cross the blood–brain barrier (Gal & Sherman 1978; Swartz et al 1990a), kynurenate was found to be poorly taken up across the blood–brain barrier (Swartz et al 1990a). Indeed, 60% of brain kynurenine has been found to originate from peripheral sources (Gal & Sherman 1978). In other work, the biosynthesis of kynurenate in brain tissue slices was shown to occur predominantly in glial cells (Turski et al 1989). Thus increased biosynthesis of kynurenate in CNS tissue following diclofenac administration may well result from increased uptake of kynurenine into CNS tissue secondary to an increase in hepatic output of kynurenine. Kynurenate was found to have an in-vitro K_i of 15 μM for the allosteric glycine site on the NMDA receptor (Kessler et al 1989). In microdialysis studies, however, the baseline concentration of kynurenate in striatal extracellular fluid was found to be 17·1 nM (Swartz et al 1990a). This is comparable with CNS tissue concentrations found in this investigation and reported by other workers (Moroni et al 1988), but is substantially lower than that required to block the glycine site on the NMDA receptor. Peripheral loading with kynurenine, however, produced up to a 37-fold increase in striatal microdialysate concentrations of kynurenate (Swartz et al 1990a). Similarly, peripheral administration of nicotinylalanine, an analogue of kynurenine that inhibits kynurenine hydroxylase and directs the flow of tryptophan metabolites toward the formation of kynurenate, doubled kynurenate concentrations, from baseline

values of ~100 nM, in hippocampal extracellular fluid and prevented audiogenic convulsions (Russi et al 1992). Connick et al (1992) have also reported that nicotinylalanine can inhibit seizures induced by leptazole or electroshock. Thus, although CNS tissue concentrations of kynurenate appear to be lower than those required to affect NMDA receptor function, under appropriate conditions, concentrations within the microenvironment of the synapse may well be high enough to inhibit NMDA receptor activation. Further studies are required examining the role of NSAIDs, in mediating antinociceptive effects through altering kynurenate concentrations, in the spinal cord and diencephalon.

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