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Changes in striatal electroencephalography and neurochemistry induced by kainic acid seizures are modified by dopamine receptor antagonists

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Abstract

We investigated the involvement of striatal dopamine release in electrographic and motor seizure activity evoked by kainic acid in the guinea pig. The involvement of the dopamine receptor subtypes was studied by systemic administration of the dopamine D_1 receptor antagonist, *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH 23390; 0.5 mg kg^{-1}), or the dopamine D_2 antagonist, (5-aminosulphonyl)-*N*-[(1-ethyl-2-pyrrolidinyl)-methyl]-2-methoxybenzamide (sulpiride, 30 mg kg^{-1}). Microdialysis and high performance liquid chromatography were used to monitor changes in extracellular levels of striatal dopamine and its metabolites, glutamate, aspartate and γ-amino-butyric acid (GABA). These data were correlated with changes in the striatal and cortical electroencephalographs and clinical signs. We found that, although neither dopamine receptor antagonist inhibited behavioural seizure activity, blockade of the dopamine D_1-like receptor with SCH 23390 significantly reduced both the 'power' of the electrical seizure activity and the associated change in extracellular striatal concentration of glutamate, whilst increasing the extracellular striatal concentration of GABA. In contrast, blockade of the dopamine D_2-like receptor with sulpiride significantly increased the extracellular, striatal content of glutamate and the dopamine metabolites. These results confirm previous evidence in other models of chemically-evoked seizures that antagonism of the dopamine D_1 receptor tends to reduce motor and electrographic seizure activity as well as excitatory amino-acid transmitter activity, while antagonism of the dopamine D_2 receptor has relatively less apparent effect. © 2001 Published by Elsevier Science B.V.

Keywords: Dopamine receptor antagonist; Microdialysis, in vivo; Electroencephalography; Kainic acid; Seizure; (Guinea pig)

1. Introduction

Kainic acid is a cyclic analogue of glutamate that evokes both electrographic and motor seizure activity when administered either intracranially or systemically into rats (Ben-Ari et al., 1980; Kleinrok and Turski, 1980; Lothman and Collins, 1981; Sperk et al., 1981). The seizures are of hippocampal origin, and spread throughout the brain, leading to a generalised *status epilepticus* (Zaczek et al., 1981). Since the demonstration of its neurotoxic properties by Olney et al. (1974), kainic acid has been widely used as a tool in neural lesioning and toxicological studies (McGeer et al, 1978). Kainic acid acts as an agonist on the kainic acid and D-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subtypes of glutamate receptors, but may also have an indirect effect on the *N*-methyl-D-aspartate (NMDA) subtype receptor (Watkins et al., 1990; Valivullah et al., 1994).

After a systemic or intracranial injection of a convulsive dose of kainic acid, distinct changes in neurotransmitter and metabolite levels can be observed in the rat brain, at times ranging from 2 to 24 h post-injection. The neurotransmitters dopamine, noradrenaline, 5-hydroxytryptamine (5-HT), γ-amino-butyric acid (GABA), glutamate, aspartate, acetylcholine, as well as their metabolites, all have been shown to increase in most brain regions, following systemic or intracranial administration of a convulsant dose of kainic acid in the rat (e.g. Kleinrok and Turski, 1980; Sperk et al., 1981, 1983; Carter et al., 1988; Carrozza et al., 1991; Bruhn et al., 1992; Fedele et al., 1993; Jin and Fredholm, 1994; Kabuto et al., 1994). The majority of these studies have monitored total cellular levels (using in vitro slice methods) rather than extracellular levels

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(using in vivo microdialysis) of neurotransmitters. The reported increase in the release of dopamine is suggested to be caused by a strong increase in the firing rate of monoaminergic neurones shortly after injection, and is unlikely to be merely a consequence of generalised seizure activity as the effect also occurs in rats that show no overt seizures (Sperk et al., 1983).

There have been relatively few studies regarding a possible role of dopamine in kainic acid-evoked epileptiform. However, clinical evidence to date suggests not only that increased dopamine activity in the brain can suppress seizure activity, but also that a reduction in dopaminergic tone is conducive to epileptogenesis (reviewed by Starr, 1996). In animal models, striatal dopaminergic activity has also been shown to play a key role in controlling the propagation of 'limbic' seizures (Chiodo, 1988; Alam and Starr, 1994). Finally, there are many examples of the pharmacological manipulation of the dopaminergic system modulating chemically induced motor and electrographic seizure activity, with dopamine D_1 receptor antagonists inhibiting seizure activity and dopamine D_2 receptor antagonists attenuating seizure activity (Al-Tajir and Starr, 1990, 1991a,b; Barone et al., 1991, 1992; Burke et al., 1990; Al-Tajir et al., 1990; Turski et al., 1991; Starr and Starr, 1993; Bourne et al., 2001).

It has long been suggested that the striatum is one of the major structures associated with seizure activity (Wada and Cornelius, 1960), and that dopamine may be important in this process, specifically in determining the seizure threshold (Trimble, 1977; Toone, 1981). However, the significance of striatal involvement in epileptiform activity remains unclear as to whether it exerts a suppressive or facilitatory effect, or is merely caught up in ictal events. This uncertainty is reflected by apparently conflicting reports of a seizure-suppressive effect of striatal stimulation on the one hand (Ono et al., 1987), and the generation of generalised motor seizures by intrastriatal injection of neuroexcitants on the other (e.g. Young et al., 1988). More recent evidence suggests that the GABAergic, striatonigral and striatopallidal pathways, which function as part of a basal ganglia feedback loop to the cortex, could play a role in the facilitation of seizure activity (Gale, 1992).

The aim of the present study was to determine the modulatory effect of subtype-specific dopamine receptor antagonists on the striatal electroencephalography and neurochemistry, in animals undergoing kainic acid-evoked seizure activity, in order to gain a better understanding of the possible involvement of the striatal dopamine receptor subtypes during epileptiform activity.

2. Materials and methods

All animal procedures were in strict accordance with Home Office (UK) guidelines and specifically licensed under the Animal (Scientific Procedures) Act, 1986. A combination of quantitative analysis of the striatal and cortical electroencephalograph (EEG) and striatal microdialysis (with the objective of measuring changes in extracellular concentrations of dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), aspartate, glutamate and GABA) was used to determine changes in the activity of transmitter-specific populations of neurones evoked by kainic acid injection, and the effects of subtype-selective dopamine receptor antagonists on these changes.

2.1. Electrode assembly

We employed an assembly with the ability to monitor concurrently the striatal EEG and extracellular neurotransmitter concentrations, via a microdialysis probe. In addition, the cortical electroencephalograph was monitored to identify regional differences in EEG activity (Bourne and Fosbraey, 2000). The construction of this apparatus was adapted from the CMA/12 Guide Probe (Carnegie Medicin, Sweden). The design was such that the two depth-electrode wires would sit in juxtaposition to the microdialysis probe when in situ.

2.2. Anaesthesia and surgery

Details of the procedure for implantation of the microdialysis/recording probe have been described in detail recently (Bourne and Fosbraey, 2000; Bourne et al., 2001). Guinea pigs weighing 250–280 g were rendered unconscious by a 5-ml kg^{-1} i.p. injection of a 1:1 mixture of Hypnorm (fentanyl citrate 0.315 mg ml^{-1}, fluanisone 10 mg ml^{-1}; Janssen Animal Health, UK); and Hypnovel (midazolam 5 mg ml^{-1}; Roche Products, UK). A guide for the CMA/12 microdialysis probe was inserted over the caudate nucleus (co-ordinates (mm) *R*: +1.3, *L*: +2.8, with respect to bregma). The tip of the guide was positioned at a depth of 4 mm below the brain surface, while the depth-electrode wires extended a further 2 mm into the striatum.

2.3. Drugs

Both *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH 23390) and (5-aminosulphonyl)-*N*-[(1-ethyl-2-pyrrolidinyl)-methyl]-2-methoxybenzamide (sulpiride) were purchased from Research Biochemicals, USA; kainic acid was obtained from Sigma, UK. Drugs were freshly prepared in sterile saline (1 mg ml^{-1}, pH 7.4). All reagents were of analytical grade.

2.4. Dialysate, EEG and behavioural sample collection

On the day of experimentation, (6–8 days post-surgery) a microdialysis probe (CMA/12; outer diameter 0.5 mm, 20,000-Da cut-off,), was inserted in such a way that the 4

mm active-membrane was exposed in the striatal region (caudate nucleus and putamen). The probe was continuously perfused with a sterile artificial cerebrospinal fluid at a flow rate of 2 μl min^{-1}. In all experiments, 40-μl dialysate samples were collected (corresponding to 20 min of perfusion).

A rodent jacket (Harvard Apparatus, USA) was attached to the animal, and to that, a multiplex radiotelemetry-transmitter (TL10M2-F50-EE implant; Data Sciences, USA) was connected. The transmitter was coupled to an electrode assembly, which allowed monitoring of two bipolar channels of electroencephalography. The sampling rate of each channel was 500 Hz.

The time to onset of pre-defined behaviours was monitored throughout the experiments to correlate with electrophysiological and dialysate data, and to determine the severity of toxicity. Observations were started prior to the administration of the dopamine receptor antagonists, to ensure that if any stereotyped behaviour was observed it would not be confused as an effect of kainic acid, and had to be observed for a minimum period of 20 s before counted as representative. The clinical signs monitored (staring, continuous fixation and absence of blinking; wet dog shakes, whole body shakes; chewing, continuous mastication and grinding of teeth; tremor, localised involuntary muscle contractions affecting normal motor behaviour with only a mild loss of posture; rearing, 'kangaroo-like' posture; circling, stereotyped movement in one direction around cage; ataxia, loss of co-ordination of muscle movements; and, convulsions, intense involuntary muscular contractions of whole body, with severe loss of posture) were those previously observed in similar studies in rats by Lothman and Collins (1981) and Ben-Ari et al. (1981).

2.5. In vivo protocol / drug treatment

An initial dose-ranging study with kainic acid (8–16 mg kg^{-1}, i.p) was used to determine a dose capable of evoking a cortical and striatal EEG *status epilepticus*, as well as behavioural clinical signs in the guinea pig, but which also allowed survival of the surgically-modified animals for 7 days post-administration. A dose of 12 mg kg^{-1} i.p. was chosen.

Following insertion of the microdialysis probe, a 120-min 'wash-out' period was started before any sampling began. Three treatment groups were used (five animals in each group): kainic acid (12 mg kg^{-1}); kainic acid (12 mg kg^{-1}) plus SCH 23390 (0.5 mg kg^{-1}); and, kainic acid (12 mg kg^{-1}) plus sulpiride (30 mg kg^{-1}). Either antagonist was administered 20 min prior to kainic acid (Bourne et al., 2001) (Fig. 1).

2.6. Neurochemical analysis

The 40-μl dialysate samples were immediately analysed by two separate systems. The analysis of dopamine, DOPAC and HVA used high performance liquid chromatography with electrochemical detection (HPLC-ECD; Kontur et al., 1984). Separation was achieved on two 100 × 3 mm Chromspher C18, 5-μm particle-size, reversed-phase glass columns (Chrompack, UK). The mobile phase (0.13 M KH_2PO_4, 0.3 mM EDTA (potassium salt), 0.5 mM sodium octane sulphonic acid and containing 10% methanol (v/v), adjusted to pH 3.0 with HCl) was used at a flow rate of 1.0 ml min^{-1}.

Separation of the amino acid neurotransmitters glutamate, aspartate and GABA was achieved on a reversed-

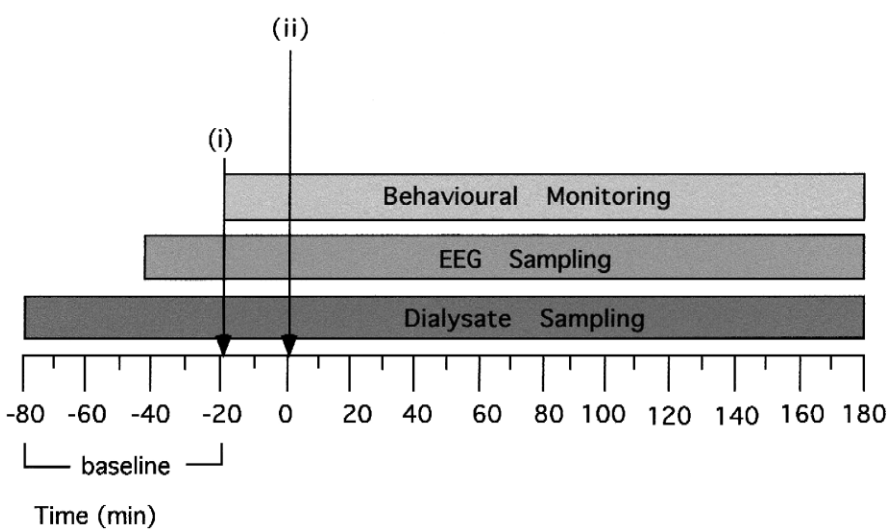

Fig. 1. Time course of neurochemical, electrophysiological and behavioural monitoring, prior to and following the administration of: (i) the dopamine D_1 receptor agonist SCH 23390 (0.5 mg kg^{-1}) or the dopamine D_2 receptor antagonist sulpiride (30 mg kg^{-1}) or saline vehicle, i.p; and (ii) kainic acid (12 mg kg^{-1}, i.p.). Baseline data were used to determine changes in the striatal and cortical electroencephalography and striatal neurochemistry following the administration of kainic acid at time 0 min. Behavioural monitoring was started at time −20 min to ensure that none of the predefined clinical signs were characteristic of kainic acid and not the dopamine antagonists.

phase Selectosil™ C18, 5 μm particle size, 100 × 3 mm, steel column. The columns were maintained at a constant 30°C, in a Gilson oven (Anachem, UK). A gradient system was used, consisting of mobile phases: (a) 0.05 M sodium acetate (buffer), 7.7 mM sodium azide (antimicrobial agent), pH 6.9 (glacial ethanoic acid), against (b) 100% methanol . The concentration of (b) was increased during the run of 25 min, starting at 17.5% and reaching a maximum 55% methanol. Two identical Gilson pumps (Anachem) delivered the flow, at a rate of 0.6 ml min^{-1}. A Gilson dynamic mixer (Anachem) performed the mixing of mobile phases. The derivatisation agent was composed of 28 mg *o*-phthalaldehyde, 1 ml methanol, 4.5 ml 0.1 M borax and 20 μl mercaptoethanol, and was replaced weekly. Preparation of each dialysate sample with 'derivatising agent' was carried out by a Gilson programmable autosampler (Anachem). The sample was mixed with 12 μl of 0.1 M borax and 8 μl of the 'derivatising agent' and the reaction allowed to proceed for 5 min before being injected onto the column. Fluorescence detection of the molecules was carried out by an ABI spectrofluorimeter (Anachem), set to excite at 225 nm and detect at 418 nm.

The basal extracellular content of dopamine, DOPAC, HVA, aspartate, glutamate and GABA in each 40-μl sample was 0.32 ± 0.02, 49.3 ± 3.2, 101.5 ± 11.2, 93.7 ± 12.2, 381.8 ± 21.9 and 24.2 ± 1.3 pmol (mean ± S.E.M., $n = 15$), respectively.

2.7. EEG analysis

The two channels of bipolar electroencephalographic data (one striatal EEG; one cortical EEG), and the reference signal were transmitted in digital form to a radiotelemetry receiver (Physiotel RLA 1020; Data Sciences) placed underneath the experimental cage. These data were then converted into analogue form and interpolated, before being fed into a CED 1401 laboratory interface (Cambridge Electronic Design, UK). The data were then reconverted to a digital signal (256-Hz, 16-bit analogue-to-digital conversion (ADC)), and subsequently analysed by a 486DX personal computer using Spike2 software (Cambridge Electronic Design). Details of the signal filtering were as in our previous studies (Bourne and Fosbraey, 2000).

2.8. Histology

Animals that survived 7 days post-administration of kainic acid, with or without antagonist, were killed by administration of an overdose of pentobarbitone sodium (Euthatal, 100 mg kg^{-1}, i.p.). Their brains were fixed for a period of at least 48 h in neutral buffered formaldehyde at room temperature. Following this, histological sections 5 μm thick were stained with Harris' haematoxylin and eosin, allowing verification of correct probe location and neuropathological examination. Animals that were humanely killed at the end of an experiment for ethical reasons were examined for correct probe location by gross dissection.

2.9. Data representation and statistical analysis

With the neurotransmitter data, the average of the three baseline samples was used to determine the pre-injection basal value. Subsequent samples were normalised as a percentage increase over the basal value, but were not corrected for probe relative recovery. The data, post-injection of the dopamine receptor antagonist/saline vehicle (0–180 min), were then summated to establish the 'total effect' (the area under the percentage change in dialysate content from basal × time curve), providing a value for evoked changes in extracellular striatal neurotransmitter concentrations for the entire experimental period. The electroencephalographic data were analysed with a 256-point 'fast Fourier transformation (FFT) algorithm', in 2 s epochs. This analysis was performed by the Spike2 software, which analysed six 2-s epochs of data every 60 s. The average of the six epochs, expressed as square microvolts, was calculated and data were expressed as 20-min totals. A normalisation was also employed for the EEG data, using the 20-min period preceding the dopamine receptor antagonist/saline vehicle administration as the basal period. As with the neurotransmitter data, a summation 'total effect' of the data for the entire experimental period was calculated (the area under the percentage change in EEG power (μV^2) from basal × time curve), which provided results of the changes in power of the striatal EEG and cortical EEG for the experimental period. Statistical analysis of the EEG and neurotransmitter 'total effect' data were performed using a Mann–Whitney *U*-test (two-tailed; with $P = 0.05$ as the limit of significance), comparing kainic acid data with kainic acid plus dopamine receptor antagonist data.

The time (min) to onset of the clinical signs, and the seizure onset defined by the EEG, were represented as the mean ± S.E.M., $n = 5$, post-injection of kainic acid, for each group. These data were subsequently analysed using a Mann–Whitney *U*-test (two-tailed; with $P = 0.05$ as the limit of significance), once again comparing kainic acid data with kainic acid plus dopamine receptor antagonist data.

3. Results

3.1. Kainic acid (12 mg kg^{-1}) treatment, $n = 5$

All animals in this treatment group ($n = 5$) exhibited both electrical and motor seizure activity, and survived 7 days post-administration of kainic acid.

The cortical and striatal EEG spike-and-wave discharges evoked by kainic acid in the guinea pig (Fig. 2) were characterised into two stages.

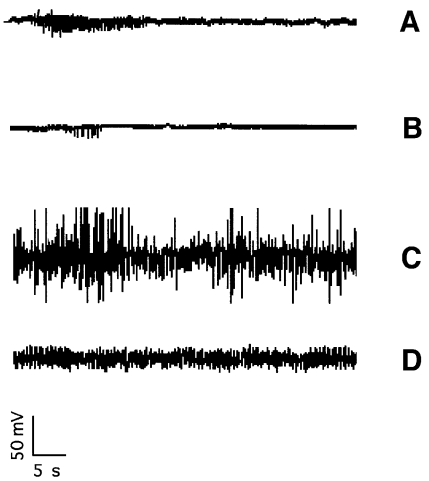

Fig. 2. Epochs of non-amplified electroencephalographic data from a single guinea pig pre- and post-administration of kainic acid (12 mg kg^{-1}) showing: (A) cortical electroencephalograph, and (B) striatal electroencephalograph, after onset of intermittent seizure activity, with long interictal periods (39 min post-administration of kainic acid); and (C) cortical electroencephalograph and (D) striatal electroencephalograph, after onset of a *status epilepticus* (106 min post-kainic acid). The spike-and-wave discharges seen in (C) and (D) remained uninterrupted (no interictal periods) for the entirety of the experiment, post-onset, with the spikes increasing in amplitude over time.

3.1.1. Intermittent seizure stage

During this phase, the EEG signal became more synchronised (Fig. 2A/B). Spike-and-wave discharges (ictal events; cortical EEG 7–11 Hz, striatal EEG 4–9 Hz) occurred but were short-lived, with the trace returning to a basal-like waveform during the interictal periods (see Table 2 for times of onset). The initial ictal events lasted 10–20 s but their duration lengthened as time progressed, and their frequency increased before progressing into a *status epilepticus*.

3.1.2. Status epilepticus

This phase was characterised by the 'fusing' together of the intermittent bursts of spike-and-wave discharges (cortical EEG 5–8 Hz, striatal EEG 4–7 Hz), to form a synchronised waveform, with spike discharges of very high amplitude (Fig. 2C/D) (see Table 2 for times of onset). Once this phase of paroxysmal activity had commenced, there was no cessation for the remainder of the recording period. This spike-and-wave discharge activity resulted in an increase (mean ± S.E.M.) in the total power (Fig. 3) of the striatal EEG (1495 ± 109% over basal) and the cortical EEG (716 ± 90% over basal).

As shown in Figs. 4 and 5, kainic acid evoked percentage increases in the total dialysate content post-administration (total effect; mean ± S.E.M.) of dopamine (413 ± 34% over basal), DOPAC (55 ± 12% over basal), HVA (22 ± 7% over basal), aspartate (14 ± 3% over basal), glutamate (261 ± 27% over basal) and GABA (55 ± 11% over basal).

All animals exhibited the behavioural signs expected from previous studies of kainic acid-induced seizures (Ta-

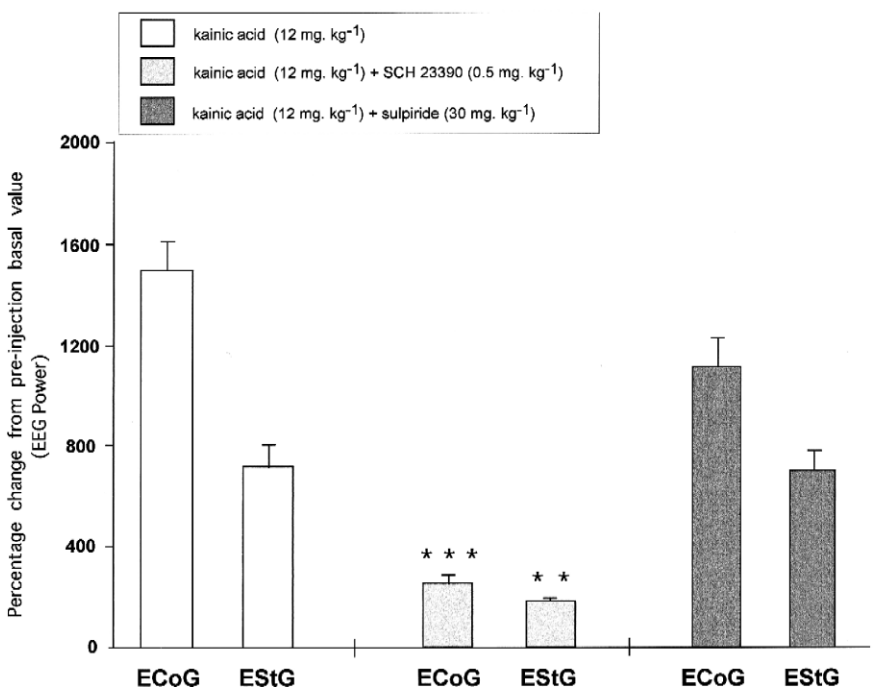

Fig. 3. Evoked changes in the electroencephalograph power 'total effect' (percentage change from baseline value for the entire post-administration of dopamine antagonist/saline vehicle period) of both the striatal electroencephalograph (EStG), using Pt/Ir depth electrodes, and cortical electroencephalograph (ECoG), using surface screws. This was calculated by summating the area under the percentage change in electroencephalographic power from basal × time curves. The power (μV^2) of the electroencephalograph was determined by fast Fourier transformation of the respective traces. Results are expressed as a percent of the respective baseline values (mean ± S.E.M., $n = 5$), the basal value being 0%. The data were analysed by a two-tailed Mann–Whitney *U*-test. $^{**}P < 0.01$; $^{***}P < 0.001$, comparing the kainic acid group with the kainic acid plus dopamine antagonist groups.

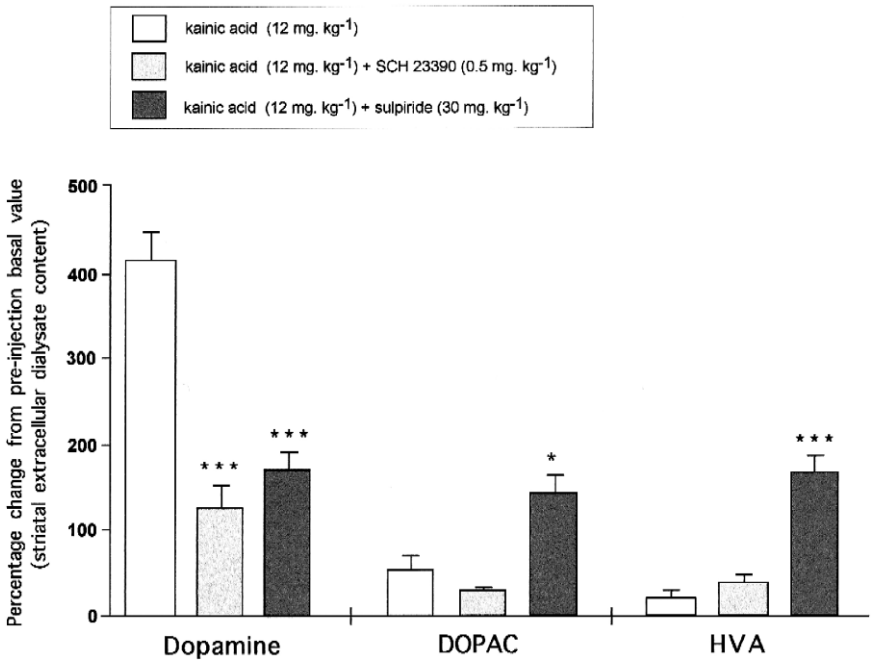

Fig. 4. Evoked changes 'total effect' (percentage change from the averaged three basal samples for the entire post-administration of dopamine antagonist/saline vehicle period) in the striatal extracelluar dialysate content of dopamine and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). This was calculated by summating the area under the percentage change in dialysate content from basal × time curves. Samples were analysed using a high performance liquid chromatography, with electrochemical detection, method. The content in each dialysate sample was calculated against a 20 pmol standard and expressed in pmol 40 μl^{-1}. Results are expressed as a percent of the respective baseline values (mean ± S.E.M., $n = 5$), the basal value being 0%. The data were analysed by a two-tailed Mann–Whitney *U*-test. $^{*}P < 0.05$; $^{***}P < 0.001$, comparing the kainic acid group with the kainic acid plus dopamine antagonist groups.

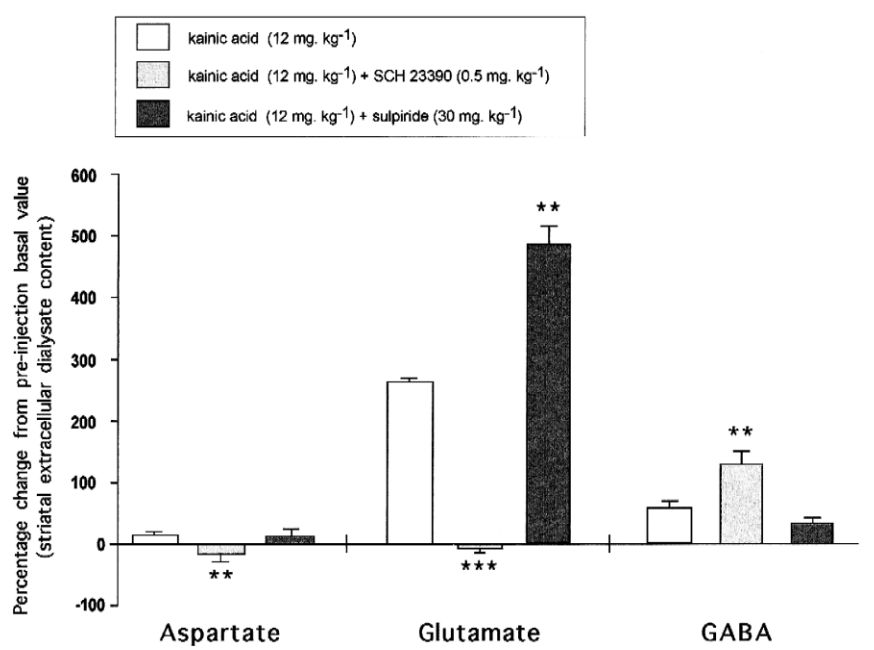

Fig. 5. Evoked changes 'total effect' (percentage change from the averaged three basal samples for the entire post-administration of dopamine receptor antagonist/saline vehicle period; the area under the curve) in the striatal extracelluar dialysate content of aspartate, glutamate and γ-amino-butyric acid (GABA). This was calculated by summating the area under the percentage change in dialysate content from basal × time curves. Samples were analysed using a high performance liquid chromatography, with fluorescence detection, method. The content in each dialysate sample was calculated against a 20 pmol standard and expressed in pmol 40 μl^{-1}. Results are expressed as a percent of the respective baseline values (mean ± S.E.M., $n = 5$), the basal value being 0%. The data were analysed by a two-tailed Mann–Whitney *U*-test. $^{**}P < 0.01$; $^{***}P < 0.001$, comparing the kainic acid group with the kainic acid plus dopamine receptor antagonist groups.

Table 1
Time to onset (min; mean ± S.E.M., $n = 5$) of pre-defined behavioural signs

Clinical sign	Time to onset (min)		
	Kainic acid (12 mg kg^{-1})	Kainic acid (12 mg kg^{-1}) + SCH 23390 (0.5 mg kg^{-1})	Kainic acid (12 mg kg^{-1}) + sulpiride (30 mg kg^{-1})
Staring	9 ± 1	8 ± 1	7 ± 1
Wet dog-shakes	22 ± 2	20 ± 1	15 ± 1[a]
Chewing	76 ± 12	78 ± 6	31 ± 3[b]
Tremor	41 ± 7	58 ± 7	29 ± 5
Rearing	30 ± 3	26 ± 5	25 ± 6
Circling	96 ± 8	111 ± 4	82 ± 2
Ataxia	211 ± 8	236 ± 4	205 ± 13
Convulsion	78 ± 5	81 ± 3	66 ± 2

[a] $P = 0.05$, according to a two-tailed Mann–Whitney *U*-test. Statistical significance was established between the kainic acid group and the kainic acid plus sulpiride group.
[b] $P = 0.01$, according to a two-tailed Mann–Whitney *U*-test. Statistical significance was established between the kainic acid group and the kainic acid plus sulpiride group.

ble 1). The first clinical sign observed was staring. Wet dog shakes and rearing were observed before the onset of convulsive episodes. The onset of cortical EEG seizure activity was seen prior to the onset of the convulsions.

Neuropathology investigation on brain tissue from animals killed 7 days post-kainic acid administration revealed mild congestion of the surface veins, accompanied by perivascular oedema and focal necrosis of the endothelial cells lining the larger intracerebral vessels. The most prominent feature however, was the degeneration of neurones in the anterior horns of the hippocampus, superio-lateral gyri of the frontal cortex, medial regions of the thalamus, inferio-lateral regions of the temporal lobes and perirhinal and entorhinal cortices. There was little evidence of mass neuronal loss. All animals were found to have correct localisation of the microdialysis probe and EEG electrodes.

3.2. Kainic acid (12 mg kg^{-1}) plus SCH 23390 (0.5 mg kg^{-1}) treatment, $n = 5$

Electrographic paroxysmal activity was observed in these animals, as seen in the kainic acid-only group (Fig. 2). The onset of intermittent seizures (Table 2) and a *status epilepticus* was not significantly different from that seen with kainic acid alone. However, there was a significant relative decrease (Fig. 3; mean ± S.E.M., $n = 5$) in the power (total effect) of the striatal EEG (255 ± 34% over basal) and the cortical EEG effect (183 ± 18% over basal), from that seen with the kainic acid group.

A significant relative decrease in the dialysate content (total effect; mean ± S.E.M., $n = 5$) of dopamine (126 ± 26 over basal) was observed (Fig. 4) in comparison to the kainic acid group, but was not observed with respect to the dopamine metabolites DOPAC (30 ± 4% over basal) and HVA (40 ± 7% over basal). A significant relative decrease was also observed (Fig. 5) in the dialysate content (total effect; mean ± S.E.M., $n = 5$) of glutamate (−8 ± 4% below basal) and a significant relative increase in the dialysate content of GABA (128 ± 21% over basal).

With this treatment group, there was no modification to the time of onset of the observed behaviours (Table 1) or neuropathology seen in the kainic acid-only group. However, differences in the duration or intensity of these behaviours cannot be excluded. All animals were found to have correct localisation of the microdialysis probe and EEG electrodes.

Table 2
Time to onset (min; mean ± S.E.M., $n = 5$) of kainic acid-evoked striatal (EStG) and cortical (ECoG) electroencephalographic intermittent seizures and a *status epilepticus*

Location	Time to onset (min)		
	Kainic acid (12 mg kg^{-1})	Kainic acid (12 mg kg^{-1}) + SCH 23390 (0.5 mg kg^{-1})	Kainic acid (12 mg kg^{-1}) + sulpiride (30 mg kg^{-1})
EStG intermittent seizures	38 ± 3	30 ± 5	35 ± 4
ECoG intermittent seizures	36 ± 5	41 ± 12	42 ± 7
EStG *status epilepticus*	104 ± 2	111 ± 8	94 ± 4
ECoG *status epilepticus*	110 ± 18	104 ± 6	89 ± 5

The data were analysed by a two-tailed Mann–Whitney *U*-test, comparing the kainic acid group with kainic acid plus dopamine antagonist groups, as well as comparing onset times at each location. No statistical significance was established.

3.3. Kainic acid (12 mg kg^{-1}) plus sulpiride (30 mg kg^{-1}) treatment, $n = 5$

The electrographic paroxysmal activity observed in these animals was comparable to that seen in the kainic acid group and the kainic acid plus SCH 23390 group (Fig. 2), consisting of two distinct stages. The onset of intermittent seizures (Table 2) and a *status epilepticus* was not significantly different from that seen with kainic acid alone. Also, there was no significant difference observed (Fig. 3) in the 'power' of the EEG (mean ± S.E.M., $n = 5$; striatal EEG 698 ± 78% over basal, cortical EEG 1109 ± 114% over basal).

There was no significant difference in the dialysate content (total effect; mean ± S.E.M., $n = 5$) of dopamine (169 ± 22 over basal; Fig. 4). However, there were significant relative increases in the dopamine metabolites DOPAC (142 ± 22% over basal) and HVA (168 ± 21% over basal) in comparison to the kainic acid group. A significant increase was also observed (Fig. 5) in the dialysate content (total effect; mean ± S.E.M., $n = 5$) of glutamate (483 ± 35% over basal), but no significant change was seen in the dialysate content of GABA (14 ± 7% over basal) or Asp (12 ± 4% over basal).

With this treatment group, the onset (Table 1; mean ± S.E.M., $n = 5$) of wet dog-shakes (15 ± 1 min) and chewing (31 ± 3 min) was observed significantly earlier than seen in the kainic acid group. There were no modifications to the neuropathology observed in the kainic acid group, and all animals were found to have correct localisation of the microdialysis probe and EEG electrodes.

4. Discussion

This study has identified significant electrophysiological (using striatal EEG and cortical EEG monitoring) and neurochemical changes (using microdialysis to monitor neurotransmitter changes) in the striatum, following the systemic administration of the chemoconvulsant kainic acid (12 mg kg^{-1}), in the guinea pig. However, unlike previous studies utilising the cholinergic agonist, pilocarpine, and the organophosphate methylphosphonofluoridate (soman), a potent inhibitor of acetylcholinesterase, neither dopamine receptor antagonist prevented the onset of seizure activity or associated neuropathology (Al-Tajir and Starr, 1990; Bourne et al., 2001). No modification to the time of onset of observed behaviours was noted, although quantitative differences in duration and or intensity were not monitored.

The occurrence of cortical EEG paroxysmal discharges with 12 mg kg^{-1} of kainic acid demonstrates that this dose was above the threshold dose required to evoke electrographic seizure activity at the presumed seizure focus (in the hippocampus) with subsequent propagation to extralimbic regions (Ben-Ari et al., 1980, 1981; Lothman and Collins, 1981). The propagation to the cortex occurs during the third stage of seizure activity, when the seizure becomes generalised, with the cortex then providing the excitatory input into the striatum via the corticostriatal pathway. There was no straightforward correlation between the onset of the behavioural signs of kainic acid-induced excitation poisoning and the onset of evoked cortical seizure activity in this present study. Such correlation has previously been observed by Lothman and Collins (1981) and Sperk et al. (1983), who studied the relationship between hippocampal EEG paroxysmal discharges and stereotyped/convulsive behaviours, in the rat. This present data showed that animals exhibited the early behavioural signs (staring, wet dog-shakes and chewing) principally during the desynchronised, interictal periods of EEG activity, seen during the intermittent seizure phase following kainic acid administration. Also, the onset of the continuous cortical and striatal spike-and-wave discharges during the *status epilepticus* preceded the motor convulsive episodes, often used to define a *status epilepticus* (Sperk et al., 1985; Wade et al., 1987). The overall course of the behaviours however, resembled that previously described for kainic acid treatments, with the more severe signs ("ataxia" and motor convulsions) being observed (several minutes) after the onset of an electrically-defined *status epilepticus*.

The dose of 12 mg kg^{-1} i.p. kainic acid in the guinea pig evoked increases in striatal extracellular dopamine, GABA, aspartate, glutamate and the dopamine metabolites DOPAC and HVA. Increases in striatal extracellular dopamine evoked by kainic acid are believed to be dose-dependent (Carter et al., 1988). The increase in striatal, extracellular dopamine observed in this study, presumably arising from its release at the terminals of the nigrostriatal dopaminergic neurones, may be due to the activation of the nigrostriatal dopaminergic neurones arising in the substantia nigra pars compacta, due to an increase in the release of excitatory transmitters in the substantia nigra which activate the nigrostriatal neurones (Heimer et al., 1982; Guevara et al., 1997). Also, the increase in striatal extracellular dopamine could be a result of activation of kainic acid release-enhancing heteroreceptors, located on the nigrostriatal dopaminergic terminals (Krebs et al., 1991). The relative decrease in dopamine in striatal dialysate seen in the kainic acid plus dopamine receptor antagonist treatment groups, in comparison with that seen with the kainic acid alone treatment group, most simply would reflect decreased activation of the nigrostriatal dopaminergic neurones. However, the relative increase in the dopamine metabolites DOPAC and HVA seen in striatal dialysate with the kainic acid plus sulpiride treatment group suggests an increase in dopamine turnover in the striatum, which may imply increased dopamine release (Michael et al., 1985). This, however, has been disputed and the increased extracellular, metabolite concentrations may rep-

resent enhanced intracellular metabolism of dopamine without release (Zetterström et al., 1988).

The increase in striatal glutamate and aspartate is believed to result in part from the activation of corticostriatal fibres. The terminals of the corticostriatal fibres also possess presynaptic kainic acid binding sites, which, when activated, can enhance glutamate/aspartate release (Chesselet, 1984). The relative decrease in the two excitatory neurotransmitters seen with the kainic acid plus SCH 23390 treatment group, from that seen with the kainic acid alone group suggests a decrease in the activation of the corticostriatal glutamatergic fibres, presumably by a decrease in the striatonigral (direct) pathway-mediated activation of the cortex, which results in decreased thalamocortical excitatory input: an obvious mechanism in which the dopamine D_1-like receptor antagonist might be working. A similar mechanism is also likely to be the explanation of the increase in glutamate observed in the kainic acid plus sulpiride treatment group, as inhibition of the dopamine D_1-associated striatopallidal (indirect) pathway results in increased thalamocortical excitatory input.

The increase in striatal GABA observed in the kainic acid alone group could be a consequence of the modulation of striatal GABAergic interneurones by other neurotransmitters, such as dopamine. Dopamine acts to enhance GABA release by activation of the presynaptic dopamine D_1 heteroreceptors located on the terminals of GABAergic striatal interneurones, which act as part of a local feedback circuit between the striatum and substantia nigra by inhibiting the activation of the striatonigral GABAergic neurones (Gale and Casu, 1981; Gale, 1992; Morari et al., 1996). However, this does not explain the increase in striatal GABA observed in the kainic acid plus SCH 23390 treatment group from that seen with the kainic acid alone group, which suggests involvement of the other dopamine subtypes or neurotransmitter heteroreceptors.

Neuropathological changes evoked by kainic acid in the guinea pig, identified from histopathological sections of appropriate central nervous system tissue, are consistent with those previously seen with 12 mg kg^{-1} in the rat, with the nuclei associated with the spread of the seizure activity (e.g. hippocampus, amygdala and the entorhinal, perirhinal and cerebral cortices) being primarily affected (Schwob et al., 1980; Ben-Ari et al., 1981). Although several mechanisms have been proposed for the neuropathological action of kainic acid, the data are consistent with the causative factor being the seizure activity per se, and not the direct excitotoxic action of the kainic acid on neuronal glutamate receptors.

In conclusion, the results generally confirm previous evidence in other models of chemically-evoked motor and electrographic seizures that antagonism of the dopamine D_1 receptor subtype tends to reduce seizure activity and excitatory amino-acid transmitter activity, while antagonism of the dopamine D_2 receptor subtype has relatively less apparent effect.

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