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SHORT COMMUNICATIONS

Convulsions and activation of epileptic foci induced by monosodium glutamate and related compounds

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It is well known that glutamate is likely to be the neurotransmitter at many vertebrate and invertebrate synapses [1–3] and L-glutamate as well as D-glutamate excite many central neurones when applied iontophoretically [4]. In addition, glutamate introduced intracerebrally causes convulsion [5, 6]. Intraperitoneal (i.p.) injections of glutamate have been shown to cause convulsions in adult and in 10-day-old rats when the dose is sufficiently high [6, 7]. It seems possible that glutamate or other excitatory amino acids could be involved in generating hyperactivity in some epileptic conditions [8, 9] and disturbances in these amino acids have been observed in epileptic foci from man and animal models [9, 10]. In the present investigation the potency of glutamate and related compounds as convulsants, and as activators of established epileptic foci was examined in adult animals.

Adult (200–250 g) Sprague–Dawley female rats were used. The compounds were administered as neutralised aqueous solutions by i.p. injection in unanaesthetised animals or by tail vein injection in lightly ether-anaesthetised animals. As found by other authors [6, 7] the excitant compounds caused convulsions after a time lag which was dose-dependent. Blood samples were taken by cardiac puncture of unanaesthetised animals before onset of fits. Focal epilepsy was induced in the animals by sprinkling cobalt metal on small circles (0.4 mm dia.) of exposed cortex located in the sensory–motor strip area [11] of the right hemisphere. Focal epilepsy set-on 5–6 days later and lasted for 10–12 days as estimated by frequency of abnormal contralateral (left hand) limb jerks, maximum epileptic activity was usually on days 8–10.

Convulsive effects. Glutamate at doses up to 20 m-moles/kg rarely had convulsive effects on adult rats as previously reported [6], but at this concentration did cause ataxia, unresponsiveness, piloerection, and accelerated, deep, breathing within 20 min of administration. At 21 m-moles/kg glutamate nearly always caused running fits and a series of 2 or 3 full grand-mal convulsions after 30–40 min, with 5–10-min periods of recovery in between (Table 1). About half the treated animals recovered fully, the rest died. Higher doses resulted in similar responses with a shortened time-lag between dose and response, the convulsions being fewer in number and always terminal. The D-isomer of glutamate appeared to be as effective as the L-isomer. Tail vein injections of glutamate caused hyper-excitability, jaw chatter, and accelerated breathing within one or two minutes, followed by running-fits and grand-mal convulsions at 3–10 min.

Table 1. Convulsions induced in adult rats by injections of amino acids and related compounds

Compound	Dose (m-moles/kg)	Time of onset of convulsion (min)
Intraperitoneal		
L-Glutamate	21	34 ± 2 (16)
D-Glutamate	21	39 ± 4 (20)
L-Aspartate	21	41 ± 3 (18)
GABA	22	none§ (5)
Taurine	14	none (6)
L-Proline	22	none (6)
Pre-injection* with GABA†	20	36 ± 4 (8)
Pre-injection* with taurine†	4	35 ± 3 (6)
Pre-injection with taurine‡	4	3 ± 0.5(6)
Folate	3	42 ± 6 (8)
2-oxoglutarate	22	31 ± 3 (8)
Succinate	22	36 ± 2 (8)
Glutarate	22	41 ± 4 (8)
Citrate	22	none (8)
Sodium chloride	22	none (8)
Tail vein		
Glutamate	10	8 ± 3 (8)
Glutamate	20	5 ± 3 (8)
Sodium chloride	22	none (8)

Intraperitoneal injections were in awake restrained control animals. Tail vein injections were given to animals under light ether anaesthesia. *Pre-injection was given at 30 min before giving glutamate† (21 m-moles/kg) or pentylenetetrazole‡ (60 mg/kg). Values are mean ± S. E. M. of number of animals tested. §'None' indicates no fits were observed in 2–3 hr.

Aspartate injected i.p. at the same dosage produced the same pattern of response as glutamate with a similar time course. Control injections of saline at equivalent molar concentrations and volumes caused none of the effects described, but the animals responded with a large increase in water intake.

Other compounds tested by i.p. injection included GABA, taurine, proline, folate, 2-oxoglutarate, succinate and citrate, all in neutral solutions at doses of 22 m-moles/kg (folate at 3.3 m-moles/kg). Of these, proline, GABA, and taurine caused little effect other than reduced movement within the cage and an increased water intake. Folate induced classical tonic-clonic fits at much lower dose (above 3.3 m-moles/kg) in 30–50 min. Citrate caused death without convulsions, presumably through its action in chelating calcium, whilst succinate and 2-oxoglutarate caused fits similar to those seen with glutamate, similar periods of delay occurring before onset. The latter two compounds were effective in only half the animals treated, whereas the effects were always seen with glutamate. Moreover, the animals survived the fits induced at 21–22 m-moles/kg whereas these were fatal with glutamate. Diarrhoea was a constant feature following injection of the keto acids. Blood levels of glutamate at the time of the first convulsions following i.p. injection of glutamate at 21 m-moles/kg were 42 ± 11 (5) mM, control being 106 ± 15 (5) μM.

No protection against fits induced by glutamate or pentylenetetrazole were afforded by injection of taurine or GABA (14–20 m-moles/kg) at 30 or 15 min prior to administration of convulsant.

Activation of epileptic foci. Sub-convulsive doses of pentylenetetrazole (20 mg/kg) activated cobalt-induced epileptic foci (Days 7–15) as judged by the appearance of readily visible and characteristic jerks of the contralateral forelimb. The limb jerk frequency was high (1–2 jerks sec), set-on in 4 min after i.p. injection and was maintained for 15–25 min before reducing in frequency and ceasing to occur. Although in qualitative terms they were identical, the jerks were often more violent than the spontaneous movement and sometimes threw the animal on its back.

Glutamate at half the subconvulsive dose (8–10 m-moles/kg) caused violent activation of the cobalt-focus after a 30–40-min delay. This settled to a regular pattern of contralateral fore-limb jerks (1–2 jerks/sec) lasting 30–40 min. The response also involved contralateral hind-limb and body trunk jerks and tremors. Saline at this and higher dose (10–22 m-moles/kg) had no effects of this kind. Another feature was activation of the 'mirror focus' which was seen as ipsilateral limb jerks occurring less frequently than the contralateral movements. The same pattern of activation occurred in animals even at days 35–40 after cobalt implantation, when all spontaneous limb jerks had ceased for 15–20 days. Doses of glutamate (21 m-moles/kg) which were convulsive in control animals were equally so in animals with active or inactive cobalt-foci. The convulsions set on with the same latency (30–40 min) as for controls but started as violent contralateral fore limb-jerks before developing into a generalized seizure. The same dose of glutamate given by stomach tube caused strong activation of the focus without developing into a general seizure, presumably due to removal of large amounts of glutamate by the liver.

It may be concluded from these and previous findings [7] that systemically administered glutamate can cause convulsions in adult as well as immature animals when given at an adequate dosage (21 m-moles/kg for rats). This effect is not specific to L-glutamate and is also seen with D-glutamate and aspartate, both of which are known to excite neurones at low concentrations [4]. The keto acids were not as consistently effective as the excitatory amino acids and caused additional symptoms such as severe diarrhoea, suggesting a more complex mode of action. In addition both succinate and 2-oxoglutarate could give rise to additional glutamate and aspartate in the blood. Inhibitory agents such as taurine and GABA did not protect against glutamate or pentylenetetrazole induced fits when administered in the same fashion.

The action of pentylenetetrazole and excitatory amino acids in causing hyperactivity of functional epileptic foci, and activation of foci which had long ceased to display spontaneous activity, as manifest as limb jerks, strongly suggests that the blood–brain barrier is defective at the focal region. This would allow entry to this region of agents present at blood concentrations at which they are normally excluded. Entry of radiolabelled folate from blood at cobalt-foci has been demonstrated by Obbens [12]. Thus entry of excitants to epileptic foci across a breach in the blood–brain barrier could be a factor controlling epileptic episodes in man and experimental animals, and also indicates a route for introduction of anticonvulsants (such as taurine [13]) which do not readily enter the brain of normal adult animals. The present results support a role for glutamate as an endogenous excitant at epileptic foci and gives further weight to the caution advised in the use of glutamate in any quantity as a food additive [6].

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