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Short communication

ARE CONVULSANT AND TOXIC PROPERTIES OF FOLATES OF THE KAINATE TYPE?

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Intra-amygdaloid injections of folic acid (FA) in rats induce behavioural, metabolic (assessed using the 2-deoxyglucose method) and neuropathological changes which, however, differ considerably from those produced by kainic acid (KA). Thus FA, in contrast to KA, does not readily induce limbic motor seizures, fails to activate the entire limbic system and does not readily reproduce the local and distant damage induced by KA, notably in the Ammon's horn of the hippocampus. The results argue against the hypothesis that KA acts at folate receptors to induce its limbic epileptic/brain damage syndrome.

Folic acid Kainic acid Amygdala Limbic seizures Brain damage

1. Introduction

Kainic acid (KA) is a potent neuroexcitatory, convulsant and neurotoxic agent which has been used extensively to produce experimentally various syndromes of clinical interest (McGeer et al., 1978). Thus, intra-amygdaloid (Iam) injections of KA produce seizures and a pattern of brain damage which is reminiscent of that seen in chronic epileptics (Ben-Ari et al., 1981). Electrophysiological (Watkins et al., 1982) and neurochemical (London et al., 1980) observations strongly suggest that KA acts at a class of receptors distinct from those mediating the excitatory action of glutamate. This raises the possibility that KA sites represent receptors for an unidentified endogenous substance. Ruck et al. (1980) have reported that folates bind to kainate sites suggesting that they may be endogenous excitotoxic analogues of KA. In keeping with this hypothesis, Olney et al. (1981) have reported that Iam injections of folates, notably folic acid (FA) itself, reproduce the limbic seizure/brain damage syndrome associated with KA. Other recent observations, however, cannot be readily reconciled with this hypothesis (Auker et al., 1981; Roberts et al., 1982; Ferkany et al., 1982). In the light of these contradictory observations, we have compared the behavioural, metabolic and histopathological sequelae produced by Iam injections of KA and FA.

2. Materials and methods

Adult male Wistar rats (250–300 g) were chronically implanted under nembutal anaesthesia with a cannula into one amygdaloid complex (the cannula did not disrupt the lateral ventricle). In some cases, a chronic catheter was placed in the jugular vein for metabolic studies which were performed by means of 2-deoxyglucose (2DG) autoradiography. KA (Sigma, 0.4–1.6 nmol dissolved in 0.1–0.4 μl phosphate buffer pH 7.4, n = 20) or FA (Sigma, 25 to 200 nmol dissolved in 0.25–1 μl sodium bicarbonate and pH adjusted with NaOH, n = 15) were injected directly into the amygdala of the unrestrained rat 1 week after surgery. Each solu-

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tion was prepared immediately before use. After injections, animals were observed for 2 h or more. For metabolic studies, [^{14}C]2DG (100 μCi/kg) was injected after the onset of motor seizures and the rats sacrificed 45 min later. The other rats were sacrificed by perfusion fixation (after 5 h to 8 days survival times) and the brains processed for histopathology using Nissl and Fink-Heimer stains. For other details, see Tremblay et al. (1983).

3. Results

As in earlier experiments, KA injections into the amygdala induced recurrent limbic motor seizures (LMS) which include successively facial and masticatory movements, forelimb clonia and rearing accompanied in severe cases by a loss of postural control (see Ben-Ari et al., 1981; Tremblay et al., 1983); a fatal status epilepticus was consistently obtained with 1.2 nmol or more Tremblay et al., 1983). The 2DG maps from KA-treated rats ($n = 8$) revealed that, in addition to the site of injection, glucose consumption was increased in several distant structures including ipsilaterally the Ammon's horn (notably the stratum pyramidale and lucidum of the CA3 field, see fig. 1H), the medio-dorsal nucleus of the thalamus and deep layers of 'limbic' cortex notably the infralimbic and prelimbic cortices (fig. 1 and Tremblay et al., 1983). A mirror focus of the metabolic increase was often present in the contralateral Ammon's horn and amygdala. Systemic KA produced a similar limbic motor syndrome accompanied by similar but bilateral metabolic alterations (fig. 1J). As extensively described elsewhere (Tremblay et al., 1983), all the structures labeled belong to or are closely related to the limbic system and, as such, are directly interconnected. KA-treated rats had a conspicuous acute necrosis and microglial proliferation at the site of injection within 24–28 h. Damage was present in distinct structures, with minor exceptions, in areas in which there had been a rise in 2DG labeling, in particular the CA3 field of Ammon's horn (fig. 2A) in which argyrophilic neurons could be detected as early as 40 min after the seizure onset, and there was partial or complete neuronal cell loss after 2–3 days (see Tremblay et al., 1983 and references therein).

In contrast to KA, independently of the dosage

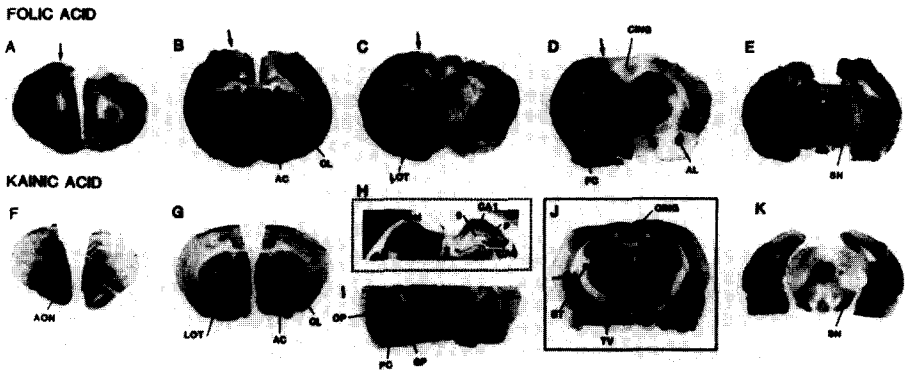

Fig. 1. Differences between the metabolic maps produced by intra-amygdaloid injection of FA (A-E, 100 nmol) and KA (F-I and K, 1 nmol) in rats; the injection site is indicated by a white arrow in D and I. The Ammon's horn pattern in (H) was obtained by putting the autoradiograph on top of the stained section from which it was obtained (see details in Tremblay et al., 1983). Photomicrograph (J) was obtained from an other case after systemic KA. All rats received 2DG after the onset of the motor seizures. Following FA, but not KA, there was an increase of labeling in the fronto-parietal cortex, up to the cingulate (Cing) cortex (arrows in A-D), globus pallidus (GP) and ventral thalamus (TV, see D). In contrast, after KA but not FA, the increase in labeling was particularly conspicuous in the prelimbic cortex (PL) and the Ammon's horn, notably the CA3 layer (H, J). Other abbreviations: AC anterior commissure; AL lateral amygdaloid nucleus; AON anterior olfactory nucleus; CL claustrum; CP caudate putamen; g granular layer of hippocampus; L lateral preoptic area; LEA lateral entorhinal area; LOT lateral olfactory tract; MD medio-dorsal thalamus; p pyramidal layer of the hippocampus; PC piriform cortex; RE reuniens; S septum; ST stria terminalis; SU subiculum.

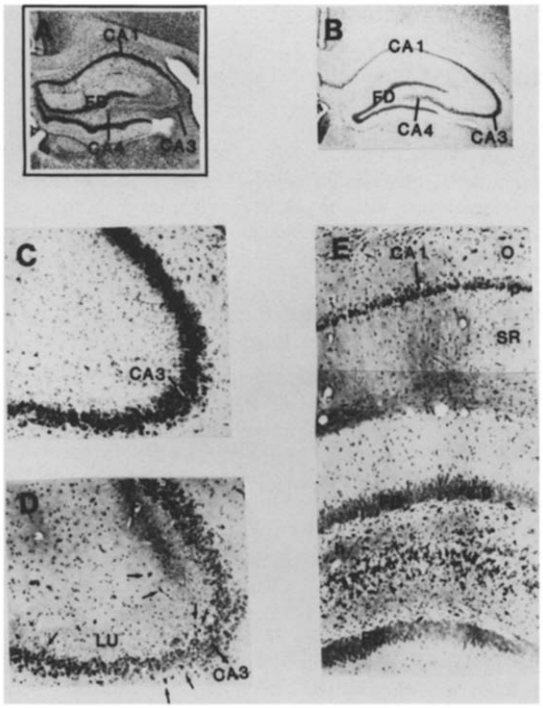

Fig. 2. Differences between the histopathological changes observed in the ipsilateral hippocampus 5 days following intra-amygdaloid injections of KA (1.2 nmol, A) and FA (100 nmol, B-E). KA produced a neuronal cell loss extending throughout the CA3 field (A, Nissl stain, magn. ×17). In contrast, FA produced little cell loss in the hippocampus (B and C, Nissl stain, magn. ×17 and 88 respectively). In alternate sections obtained from the same case as in B and C and stained with Fink-Heimer method, argyrophilic neurons were observed in the hilus and CA1 (E, magn. ×88) but not in CA3, with the exception of a few positive neurons located outside the pyramidal layer (arrows in D, magn. ×88). Abbreviations: h hilus; FD fascia dentata; LU stratum lucidum; O stratum oriens; R stratum radiatum; see also fig. 1.

used (25–200 nmol), FA injections into amygdala seldom induced LMS and, when present, LMS were generally aborted. In contrast, FA produced motor seizures which were characterized by twisting of the body with head clonia or jerking movements of the paws contralaterally to the site of injection; stereotyped displacements and head jerkings from side to side, gnawing, wet-dog shakes and contralateral circling were present. The FA motor syndrome decreased after 2–3 h and fatal status was not induced (even with 200 nmol). In contrast to KA, FA (n = 4) consistently caused an increase in 2DG labeling in the entire piriform lobe and fronto-parietal convexity (fig. 1A-D), the ventral thalamus (fig. 1D) as well as the globus pallidus (fig. 1C) and subthalamic area (not shown). In further contrast to the effect of KA, several major limbic structures were not or poorly affected, notably the rostral Ammon's horn (fig. 1C) as well as the infra- and prelimbic cortices (fig. 1A), and contralateral foci were only present in the substantia nigra and hippocampus in one case fig. 1D). At the site of injection, doses of 50 nmol or more produced local damage in the amygdala, although this was not as extensive and not associated with massive microglial proliferation as on KA injection. FA also produced 'distant' damage; this, however, differed considerably from the KA pattern. Thus, major limbic structures – notably the pyramidal layer of CA3 – were usually not damaged after FA; when present in the Ammon's horn, the pathological changes preferentially affected the hilus area and CA1 rather than CA3 (fig. 2B-E; compare with the typical effect of KA shown in A). In contrast, FA produced damage in the ventral thalamus and disseminated neuronal cell loss in extrapyramidal structures (notably the globus pallidus). Furthermore, the most conspicuous 'distant' damage produced by FA was in the piriform and the fronto-parietal cortices. In the former, the damage consisted in complete necrosis extending throughout all the layers, whereas after Iam KA the damage, when present, was restricted to neurons of the deeper layers. In the fronto-parietal cortex, the damage was characterized by the presence of argyrophilic neurons in the superficial layers and bands of status spongiosus containing ischemic cell changes with or without incrustations; this was not seen after Iam KA. Furthermore, the time course of the brain damage (local and distant) induced by FA was slower than that of damage produced by KA (see also Roberts et al., 1982).

4. Discussion

Our results showing that Iam FA, even in large doses (100–200 nmol), could not readily reproduce

the effects of much smaller doses of Iam KA (1.2 nmol) but induced other behavioural, metabolic and neuropathological changes are not in agreement with those of Olney and his group (1981) who used similar FA dosages. Although the reasons for this discrepancy are not clear there are now several electrophysiological, (Auker et al., 1982), biochemical (Roberts et al., 1981; Ferkany et al., 1982) or neuropathological (Ferkany et al., 1982) observations indicating that folates fail to mimic KA. There are now also several lines of evidence to suggest that FA (or derivatives) does not compete for [^{3}H]KA binding sites in several brain regions including the striatum (Ferkany et al., 1982), amygdala, hippocampus and fronto-parietal cortex (Tremblay et al., submitted). Therefore, we argue against the hypothesis that Iam KA acts at FA receptors to produce its strong limbic epileptic/brain damage syndrome.

In our experiments, the distribution and type of brain damage following FA (notably in the fronto-parietal cortex) is reminiscent of that produced in primate and subprimate species by a variety of conditions associated with severe anoxia and (or) ischemia, such as severe reduction in cerebral perfusion pressure, intra-carotid air embolism or atmospheric decompression; this has been extensively investigated by Brierley (1971). Thus, we think that the mechanisms of the convulsions and brain damage produced by FA merit further investigation, in particular with regard to the selective vulnerability of brain structures to hypoxia and ischemia.

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